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(54) **Plants with modified stamen cells**

Pflanzen mit modifizierten Staubblattzellen

Plantes avec des cellules d'étamine modifiées

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

This invention relates to a male-sterile plant and to its reproduction material (e.g., seeds), in which the cells are transformed so that a foreign DNA sequence is stably integrated into their nuclear genome. The male-sterile plants of this invention contain, in all of their cells, a foreign DNA sequence which contains at least one first foreign DNA (hereinafter the "male-sterility DNA") that: 1) encodes a first RNA or protein or polypeptide which, when produced or overproduced in a stamen cell of the plant, disturbs significantly the metabolism, functioning and/or development of the stamen cell; and 2) is in the same transcriptional unit as, and under the control of, a first promoter which is capable of directing expression of the male-sterility DNA selectively in stamen cells of the plant, provided that, if the first promoter is a promoter which directs expression of the male-sterility DNA selectively in microspore and/or pollen cells, the nuclear genome of the cells of the plant is homozygous. In particular, this invention relates to such a nuclear male-sterile plant and its reproduction material, in which the foreign DNA sequence of this invention is a foreign chimaeric DNA sequence that can also contain at least one second foreign DNA (the "marker DNA") that: 1) encodes a second RNA or protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the entire plant easily separable from other plants that do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; 2) is in the same transcriptional unit as, and under the control of, a second promoter which is capable of directing expression of the marker DNA in at least the specific tissue or the specific cells of the plant; and 3) is in the same genetic locus of the nuclear genome of the cells of the plant as the male-sterility DNA.

This invention also relates to a foreign chimaeric DNA sequence that contains at least one male-sterility DNA under the control of the first promoter, provided that the first promoter is not a microspore and/or pollen specific promoter, and that can also contain, adjacent to the male-sterility DNA, at least one marker DNA under the control of the second promoter.

This invention further relates to a vector that contains the foreign DNA sequence of this invention and is suitable for the transformation of plant cells, whereby the foreign DNA sequence is stably integrated into the nuclear genome of the cells, provided that the first promoter is not a microspore and/or pollen specific promoter,

This invention still further relates to cells of a plant and to plant cell cultures, the nuclear genomes of which are transformed with the foreign DNA sequence, provided that, if the first promoter is a promoter which directs expression of the male-sterility DNA selectively in microspore and/or pollen cells, the nuclear genome of the cells is homozygous. This invention yet further relates to a process for producing a nuclear male-sterile plant and its reproduction material and its cell cultures containing the foreign DNA sequence in which the male-sterility DNA: 1) is under the control of the first promoter and optionally in the same genetic locus as the marker DNA under the control of the second promoter; 2) is stably integrated into the nuclear genome of the plant's cells; and 3) can be expressed selectively in stamen cells of the plant in the form of the first RNA, protein or polypeptide.

The invention further relates to a process for producing hybrid seeds, which grow into hybrid plants, by crossing: 1) the male-sterile plant of this invention which includes, in its nuclear genome, the marker DNA, preferably encoding a protein conferring a resistance to a herbicide on the plant; and 2) a male-fertile plant without the marker DNA in its genome. This invention particularly relates to such a process for producing hybrid seeds on a commercial scale, preferably in a substantially random population, without the need for extensive hand-labor. This invention still further relates to a tapetum-specific promoter from a plant genome. This promoter can be used as the first promoter in the foreign DNA sequence of this invention for transforming the plant to render it nuclear male-sterile.

Background of the Invention

Hybridization of plants is recognized as an important process for producing offspring having a combination of the desirable traits of the parent plants. The resulting hybrid offspring often have the ability to outperform the parents in different traits, such as in yield, adaptability to environmental changes, and disease resistance. This ability is called "heterosis" or "hybrid vigor". As a result, hybridization has been used extensively for improving major crops, such as corn, sugarbeet and sunflower. For a number of reasons, primarily related to the fact that most plants are capable of undergoing both self-pollination and cross-pollination, the controlled cross-pollination of plants without significant self-pollination, to produce a harvest of hybrid seeds, has been difficult to achieve on a commercial scale.

In nature, the vast majority of crop plants produce male and female reproductive organs on the same plant, usually in close proximity to one another in the same flower. This favors self-pollination. Some plants, however, are exceptions as a result of the particular morphology of their reproductive organs which favors cross-pollination. These plants produce hybrid offspring with improved vigor and adaptability. One such morphology in Cannabis ssp. (hemp) involves male and female reproduction organs on separate plants. Another such morphology in Zea mays (corn) involves male and female reproductive organs on different parts of the same plant. Another such morphology in Elaeis guineensis (oilpalm) involves male and fertile female gametes which become fertile at different times in the plant's development.

Some other plant species, such as Ananas comosus (pineapple), favor cross-pollination through the particular

physiology of their reproductive organs. . Such plants have developed a so-called "self-incompatibility system" whereby the pollen of one plant is not able to fertilize the female gamete of the same plant or of another plant with the same genotype.

Some other plant species favor cross-pollination by naturally displaying the so-called genomic characteristic of "male sterility". By this characteristic, the plants' anthers degenerate before pollen, produced by the anthers, reach maturity. See: "Male-Sterility in Higher Plants", M.L.H. Kaul, 1987, in: Monographs on Theoretical and Applied Genetics 10, Edit. Springer Verlag. Such a natural male-sterility characteristic is believed to result from a wide range of natural mutations, most often involving recessive deficiencies, and this characteristic can not easily be maintained in plant species that predominantly self-pollinate, since under natural conditions, no seeds will be produced.

There are four main types of male sterility observed in nature. All four types of male sterility are used in commercial breeding programs to ensure that there is cross-pollination to produce hybrid seed for crops such as corn, sugarbeet, oilseed rape and sunflower.

One type of male sterility is nuclear encoded and is believed to be inherited as a recessive allele. For breeding purposes, a recessive male-sterile parent plant is maintained by crossing it with a heterozygous male-fertile plant that also includes the recessive male-sterility allele, so that the offspring are 50% recessive male-sterile plants. The other 50% are male-fertile plants that have to be rogued out in outcrossing programs which can only be done efficiently if the recessive male-sterility allele is segregated together with a selectable or screenable-marker. In US patent 4,727,219, a procedure is described for the use of recessive male sterility for the production of hybrid maize.

A second type of male sterility is nuclear encoded but inherited as a dominant allele. An advantage of dominant male sterile plants, as compared to recessive male sterile plants, is that the dominant male-sterile plants can be maintained through crossing with a male-fertile plant, to produce offspring that are 50% dominant male-sterile plants. The usefulness of this dominant nuclear male-sterile plant is, however, limited because its dominant male-sterility allele is in most cases not tightly linked (i.e., within the same genetic locus) to a selectable or screenable marker.

A third type of male sterility is cytoplasmatically encoded. In most cases, the cytoplasmic code is in the mitochondrial genome of the plant, and only in a few cases is the code in the chloroplast genome of the plant. The inheritance of cytoplasmatically encoded male sterility does not follow Mendelian rules but rather depends on cytoplasmic factors. The offspring obtained from crosses between cytoplasmic male-sterile plants and male-fertile plants all carry the cytoplasmic male-sterility gene and are therefore sterile. As a result, the offspring of plants of this type are only of commercial value if the economic product of the offspring is not for use as seed but rather for plants such as ornamentals and sugarbeet.

A fourth type of male sterility is the result of a combination of both nuclear encoded male sterility and cytoplasmatically encoded male sterility. The male sterility-inducing nuclear alleles are usually recessive, and only plants that contain the male-sterility cytoplasmic allele and that are homozygous for the male sterility-inducing nuclear allele are phenotypically male sterile. In this type of plant, corresponding dominant male fertility-inducing alleles or "restorers of fertility" produce a male-fertile phenotype. As a result, the male-sterile offspring of this type of plant can be made male-fertile by pollinating the male-sterile plants with pollen containing the restorers of fertility. As a result, the offspring of plants of this type are of commercial value where the economic product is seed, that is for plants such as corn, sorghum and sunflower.

Typically, hybrid seed production has been accomplished by the large scale planting of cytoplasmic male-sterile plants and male-fertile plants and by somehow (e.g., with a distinctive marker) preventing the resulting hybrid seeds from becoming mixed with non-hybrid seeds. According to U.S. patent no. 3,842,538, hybrid seeds are tediously separated from non-hybrid seeds on the basis of color. According to U.S. patent no. 4,351,130, the problem of separating hybrid seeds from non-hybrid seeds is avoided by using short male-sterile plants and tall male-fertile plants and then destroying the tall male-fertile plants after pollination. According to U.S. patents 4,658,085, 4,517,763 and 4,658,084, cytoplasmic male-sterile plants are provided with a herbicide tolerance absent from the male-fertile plants which are destroyed with the herbicide after pollination. According to U.S. patent no. 4,305,225, male-sterile rice plants are sprayed with a growth hormone (e.g., gibberellin) in order to cause fuller emergence of flower-bearing panicles from rice leaf sheaths, thereby increasing the ability of the flowers to receive pollen from male-fertile plants.

In all such processes for producing hybrid seeds from male-sterile plants, ways have been sought for simply and inexpensively obtaining on a commercial scale: 1) high hybrid seed production from each male-sterile plant; 2) a hybrid seed population that results almost exclusively from pollen of male-fertile plants and eggs of male-sterile plants and is substantially free of non-hybrid seeds from male-fertile plants; 3) easy production of both the male-sterile and male-fertile plants; and 4) the virtually complete removal or destruction of either the male-fertile plants after they have pollinated the male-sterile plants or the selective separation of non-hybrid seeds, produced by the male-fertile plants, from the hybrid seeds produced by the male-sterile plants.

By genetic engineering, efforts have been made to provide more useful male-sterility characteristics in plants. See for example:

Advanced Genetic Sciences, EP-A-O 198 288 which describes inter alia: the transformation of nuclear male-sterile plants with DNA encoding a marker; and the use of cross-breeding techniques to produce plants in which the marker DNA is closely linked to endogenous DNA encoding nuclear male sterility.

Paladin Hybrids, EP-A-0 329 308 which describes inter alia: the transformation of plants with a foreign chimaeric DNA sequence comprising:

a) a pollen specific promoter hooked to a DNA which codes for a cytotoxin;
b) a promoter hooked to an antisense DNA fragment of a gene "critical for pollen formation or function";
c) a promoter of a gene "critical for pollen formation or function" hooked to an antisense DNA fragment of a gene which is also present in the plant and which confers resistance to a herbicide or physiological stress.

Summary of the Invention

The invention provides a male-sterile plant containing a foreign DNA incorporated in the nuclear genome of its cells, wherein the foreign DNA comprises

a) a male-sterility DNA encoding a first RNA, protein or polypeptide, capable when produced in stamen cells of the plant, of killing or disabling them to prevent the production of fertile male gametes; and
b) a first promoter which directs gene expression selectively in stamen cells of the plant, the male sterility DNA being in the same transcriptional unit as, and under the control of the first promoter,

provided that, if the first promoter is a promoter which directs expression of the male-sterility DNA selectively in microspore and/or pollen cells, the nuclear genome of the cells of the plant is homozygous.

The first promoter preferably directs expression in anther cells, particularly in tapetum or anther epidermal cells. The male-sterility DNA preferably encodes a ribonuclease, such as barnase.

Further in accordance with this invention a DNA, such as nuclear DNA of a cell of a plant, is provided which contains a first chimeric DNA which comprises a) a male-sterility DNA encoding a first RNA, protein or polypeptide, capable when produced in stamen cells of a plant, of killing or disabling them to prevent the production of fertile male gametes; and b) a first promoter which directs gene expression selectively in stamen cells of the plant, the male sterility DNA being in the same transcriptional unit as, and under the control of the first promoter, provided that the first promoter is not a microspore- and/or pollen specific promoter.

Preferably such first promoter directs expression in anther cells, particularly in tapetum or anther epidermal cells. The male-sterility DNA preferably encodes a ribonuclease such as barnase.

The DNA of this invention can also contain a second chimeric DNA which comprises :

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the plant easily separable from other plants which do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; and
(d) a second promoter capable of directing expression of the marker DNA at least in the specific tissue or specific cells; the marker DNA being in the same transcriptional unit as, and under the control of, the second promoter.

The marker DNA is preferably a herbicide resistance gene.

The DNA of this invention can further contain at least one additional DNA encoding a transit peptide capable of transporting the first protein or polypeptide or the second protein or polypeptide into a chloroplast or mitochondria of a plant cell in which the foreign chimaeric DNA sequence is expressed in its cytoplasm.

Further in accordance with this invention are provided: a cell of a plant, a plant cell culture, a plant, and a seed of a plant containing the DNA of this invention; and a pair of parent plants comprising a) a male-sterile parent plant containing the DNA of this invention, and b) a male-fertile parent plant.

Further in accordance with this invention a process is provided to produce a male-sterile plant and reproduction materials , e.g. seeds, by transforming a cell of a plant with a DNA comprising the male-sterility DNA under control of the first promoter, regenerating the male-sterile plant from the transformed plant cell, and optionally, obtaining the reproduction material or progeny of the male-sterile plant.

Still further in accordance with this invention a process is provided for producing a seed of a plant which is a seed-forming and male-sterile plant, which comprises:

- cross-pollinating i) male-sterile, seed-forming plants of this invention containing, incorporated into the nuclear genome of all of their cells, the male-sterility DNA under control of the first promoter and a marker DNA, which is a

gene conferring resistance to a herbicide or a gene encoding a modified target enzyme for a herbicide, under control of the second promoter, and ii) male-fertile plants without the marker DNA and the second promoter,

- applying the herbicide to the plants for eliminating male-fertile plants,
- obtaining seeds of the pollinated male-sterile plants.

Still further in accordance with this invention a process is provided to maintain an inbred line of male-sterile, seed-forming plants of this invention containing, incorporated into the nuclear genome of all of their cells in heterozygous form, the male-sterility DNA under control of the first promoter and a marker DNA, which is a gene conferring resistance to a herbicide or a gene encoding a modified target enzyme for the herbicide, under control of the second promoter, the process comprising :

- i) cross-pollinating male-sterile plants of the inbred line, and ii) male-fertile plants of the inbred line without the marker DNA and the second promoter, and, after cross-pollination,
- obtaining seeds from the male-sterile plants,
- growing these seeds into plants and
- applying the herbicide to the plants for eliminating male-fertile plants.

Still further in accordance with this invention is provided a tapetum specific promoter.

Description of the Invention

In accordance with this invention, a male-sterile plant is produced from a single cell of a plant by transforming the plant cell in a well known manner to stably insert, into the nuclear genome of the cell, the foreign DNA sequence of this invention. The foreign-DNA sequence comprises at least one male-sterility DNA that is under the control of, and fused at its 5' end to, the first promoter and is fused at its 3' end to suitable transcription regulation signals (including a polyadenylation signal). Thereby, the first RNA, protein or polypeptide is produced or overproduced selectively in stamen cells of the plant so as to render the plant male-sterile. Preferably, the foreign DNA sequence also comprises at least one marker DNA that is under the control of, and is fused at its 5' end to, the second promoter and is fused at its 3' end to suitable transcription regulation signals (including a polyadenylation signal). The marker DNA is preferably in the same genetic locus as the male-sterility, whereby the second RNA, protein or polypeptide is produced in at least the specific tissue or specific cells of the plant so that the plant can be easily distinguished and/or separated from other plants that do not contain the second RNA, protein or polypeptide in the specific tissue or specific cells. This guarantees, with a high degree of certainty, the joint segregation of both the male-sterility DNA and the marker DNA into offspring of the plant.

The cell of a plant (particularly a plant capable of being infected with Agrobacterium) is preferably transformed in accordance with this invention, using a vector that is a disarmed Ti-plasmid containing the foreign DNA sequence and carried by Agrobacterium. This transformation can be carried out using procedures described, for example, in European patent publications 0,116,718 and 0,270,822. Preferred Ti-plasmid vectors contain the foreign DNA sequence between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example, in European patent publication 0,223,247), pollen mediated transformation (as described, for example, in European patent publication 0,270,356, PCT publication WO85/01856, and European patent publication 0,275,069), in vitro protoplast transformation (as described, for example, in US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in European patent publication 0,067,553, and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475).

Preferably, a nuclear male-sterile plant of this invention is provided by transforming a plant cell with a disarmed Ti-plasmid vector containing the foreign DNA sequence with both a male-sterility DNA under the control of a first promoter and a marker DNA under the control of a second promoter. The marker DNA can be upstream or downstream of the male-sterility DNA in the Ti-plasmid vector, but preferably, the two are adjacent to one another and are located between the border sequences or at least located to the left of the right border sequence of the Ti-plasmid vector, so that they are properly transferred together into the nuclear genome of the plant cell. However, if desired, the cell can initially be transformed with a foreign DNA sequence containing a male-sterility DNA and a first promoter and can subsequently be transformed with a marker DNA and a second promoter, inserted into the same genetic locus in the cell's nuclear genome as the male-sterility DNA. Suitable vectors for this purpose are the same as those discussed above for transforming cells with the foreign DNA sequence. The preferred vector is a disarmed Ti-plasmid vector.

The selection of the male-sterility DNA is not critical. A suitable male-sterility DNA can be selected and isolated in a well-known manner, so that it encodes the first RNA, protein or polypeptide which significantly disturbs the proper metabolism, functioning and/or development of any stamen cell in which the male-sterility DNA is expressed, preferably

leading thereby to the death of any such stamen cell. Preferred examples of male-sterility DNAs encode: RNases such as RNase T1 (which degrades RNA molecules by hydrolyzing the bond after any guanine residue) and Barnase; DNases such as an endonuclease (e.g., EcoRI); or proteases such as a papain (e.g., papain zymogen and papain active protein).

Other examples of male-sterility DNAs encode enzymes which catalyze the synthesis of phytohormones, such as: isopentenyl transferase which is an enzyme that catalyzes the first step in cytokinin biosynthesis and is encoded by gene 4 of Agrobacterium T-DNA; and the enzymes involved in the synthesis of auxin and encoded by gene 1 and gene 2 of Agrobacterium T-DNA. Yet other examples of male-sterility DNAs encode: glucanases; lipases such as phospholipase A_2 (Verheij et al (1981) Rev. Biochem. Pharmacol. 91, 92-203); lipid peroxidases; or plant cell wall inhibitors. Still other examples of male-sterility DNAs encode proteins toxic to plants cells, such as a bacterial toxin (e.g., the A-fragment of diphtheria toxin or botulin).

Still another example of a male-sterility DNA is an antisense DNA which encodes a strand of RNA complementary to a strand of RNA that is naturally transcribed in the plant's stamen cells under the control of an endogenous promoter as described, for example, in European patent publication 0,223,399. Such an antisense DNA can be transcribed into an RNA sequence capable of binding to the coding and/or non-coding portion of an RNA, naturally produced in the stamen cell, so as to inhibit the translation of the naturally produced RNA. An example of such an antisense DNA is the antisense DNA of the TA29 gene (described in Example 2) which is naturally expressed, under the control of the TA29 promoter, in tapetum cells of the anthers of plants.

A further example of a male-sterility DNA encodes a specific RNA enzyme (i.e., a so-called "ribozyme"), capable of highly specific cleavage against a given target sequence, as described by Haseloff and Gerlach (1988) Nature 334, 585-591. Such a ribozyme is, for example, the ribozyme targeted against the RNA encoded by the TA29 gene.

Still other examples of male-sterility DNAs encode products which can render the stamen cells susceptible to specific diseases, such as fungus infections. Such a male-sterility DNA can be used in a plant wherein all other cells, in which the male-sterility DNA is not expressed, are resistant to the specific disease.

By "foreign" with regard to the foreign DNA sequence of this invention is meant that the foreign DNA sequence contains a foreign male-sterility DNA and/or a foreign first promoter. By "foreign" with regard to a DNA, such as a male-sterility DNA and a first promoter, as well a marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant that such a DNA is not in the same genomic environment in a plant cell, transformed with such a DNA in accordance with this invention, as is such a DNA when it is naturally found in the cell of the plant, bacteria, animal, fungus, virus, or the like, from which such a DNA originates. This means, for example, that a foreign male-sterility DNA or marker DNA can be: 1) a nuclear DNA in a plant of origin; 2) endogenous to the transformed plant cell (i.e., from a plant of origin with the same genotype as the plant being transformed); and 3) within the same transcriptional unit as its own endogenous promotor and 3' end transcription regulation signals (from the plant of origin) in the foreign DNA sequence of this invention in the transformed plant cell; but 4) inserted in a different place in the nuclear genome of the transformed plant cell than it was in the plant of origin so that it is not surrounded in the transformed plant cell by the genes which surrounded it naturally in the plant of origin. A foreign male-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a different (i.e., not its own) endogenous promotor and/or 3' end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign male-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a heterologous promotor and/or 3' end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign male-sterility or marker DNA can also, for example, be heterologous to the transformed plant cell and in the same transcriptional unit as an endogenous promotor and/or 3' transcription regulation signals (e.g., from the nuclear genome of a plant with the same genotype as the plant being transformed) in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. An example of a foreign male-sterility DNA could come from the nuclear genome of a plant with the same genotype as the plant being transformed and encode a catalytic enzyme, such as a protease or ribonuclease, that is endogenous to stamen cells of the plant being transformed, so that the enzyme is overproduced in transformed stamen cells in order to disturb significantly their metabolism, functioning and/or development. Preferably, the male-sterility DNA and the marker DNA are each heterologous to the plant cell being transformed.

By "heterologous" with regard to a DNA, such as a male-sterility DNA, a first promoter, a marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant that such a DNA is not naturally found in the nuclear genome of cells of a plant with the same genotype as the plant being transformed. Examples of heterologous DNAs include chloroplast and mitochondrial DNAs obtained from a plant with the same genotype as the plant being transformed, but preferred examples are chloroplast, mitochondrial, and nuclear DNAs from plants having a different genotype than the plant being transformed, DNAs from animal and bacterial genomes, and chromosomal and plasmidial DNAs from fungal and viral genomes.

By "chimaeric" with regard to the foreign DNA sequence of this invention is meant that at least one of its male-

sterility DNAs: 1) is not naturally found under the control of its first promoter for the one male-sterility DNA; and/or 2) is not naturally found in the same genetic locus as at least one of its marker DNAs. Examples of foreign chimaeric DNA sequences of this invention comprise: a male-sterility DNA of bacterial origin under the control of a first promoter of plant origin; and a male-sterility DNA of plant origin under the control of a first promoter of plant origin and in the same genetic locus as a marker DNA of bacterial origin.

So that the male-sterility DNA is expressed selectively in stamen cells of a plant, it is preferred that the first promoter, which controls the male-sterility DNA in the foreign DNA sequence, be a promoter capable of directing gene expression selectively in stamen cells of the plant. (By "stamen" is meant the organ of the flower that produces the male gamete and that includes an anther and a filament). Such a stamen-specific promoter can be an endogenous promoter or an exogenous promoter and can be from the nuclear genome or from the mitochondrial or chloroplast genome of a plant cell. In any event, the first promoter is foreign to the nuclear genome of the plant cell, being transformed. Preferably, the first promoter causes the male-sterility DNA to be expressed only in anther, pollen or filament cells, especially in tapetum or anther epidermal cells. The first promoter can be selected and isolated in a well known manner from the species of plant, to be rendered male-sterile, so that the first promoter directs expression of the male-sterility DNA selectively in stamen cells so as to kill or disable the stamen and render the plant incapable of producing fertile male gametes. The first promoter is preferably also selected and isolated so that it is effective to prevent expression of the male-sterility DNA in other parts of the plant that are not involved in the production of fertile pollen, especially in female organs of the plant. For example, a suitable endogenous stamen-specific first promoter can be identified and isolated in a plant, to be made male-sterile, by:

1. searching for an mRNA which is only present in the plant during the development of its stamen, preferably its anthers, pollen or filament;
2. isolating this stamen-specific mRNA;
3. preparing a cDNA from this stamen-specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the stamen-specific mRNA; and then
5. identifying the portion of the plant genome that is upstream (i.e., 5') from the DNA coding for the stamen-specific mRNA and that contains the promoter of this DNA.

Examples of such first promoters are the TA29 promoter, the TA26 promoter and the TA13 promoter, hereinafter described in the Examples, which have been isolated from tobacco and are tapetum-specific promoters. Another tapetum-specific first promoter from another plant species can be isolated from its genome, using the TA29, TA26 or TA13 gene as a probe as in step 4, above. Under hybridizing conditions, such a probe will hybridize to DNA coding for a tapetum-specific mRNA in a mixture of DNA sequences from the genome of the other plant species (Maniatis et al (1982) Molecular Cloning, A Laboratory Manual. Ed. Cold Spring Harbor Laboratory). Thereafter, as in step 5 above, the other tapetum-specific first promoter can be identified.

If more than one male-sterility DNA is present in the foreign DNA sequence of this invention, all the male-sterility DNAs can be under the control of a single first promoter, but preferably, each male-sterility DNA is under the control of its own separate first promoter. Where a plurality of male-sterility DNAs are present in the foreign DNA sequence, the male-sterility DNA also can encode the same or different first RNA(s), polypeptide(s) and protein(s). For example, when the male-sterility DNA encodes an RNase such as RNase T1, it preferred that at least 3, particularly 4 to 6, copies of the male-sterility DNA and its first promoter be provided in the foreign DNA sequence. In any event, all the male-sterility DNA(s) and their first promoter(s) are preferably adjacent to one another in the foreign DNA sequence and in any vector used to transform plant cells with the foreign DNA sequence.

The selection of the marker DNA also is not critical. A suitable marker DNA can be setected and isolated in a well known manner, so that it encodes a second RNA, protein or polypeptide that allows plants, expressing the marker DNA, to be easily distinguished and separated from plants not expressing the second RNA, protein or polypeptide. Examples of marker DNAs encode proteins that can provide a distinguishable color to plant cells, such as the Al gene encoding dihydroquercetin-4-reductase (Meyer et al (1987) Nature 330, 677-678) and the glucuronidase gene (Jefferson et al (1988) Proc. Natl. Acad. Sci. USA ("PNAS") 83, 8447), or that provide a specific morphological characteristic to the plant such as dwarf growth or a different shape of the leaves. Other examples of marker DNAs confer on plants: disease or pest resistance such as is provided by a gene encoding a Bacillus thuringiensis endotoxin conferring insect resistance as described in European patent publication 0,193,259 or a gene encoding a bacterial peptide that confers bacterial resistance as described in European patent publication 0,299,828.

Preferred marker DNAs encode second proteins or polypeptides inhibiting or neutralizing the action of herbicides such as: the sfr gene and the sfrv gene encoding enzymes conferring resistance to glutamine synthetase inhibitors such as bialaphos and phosphinothricin as described in European patent publication 0,242,246; genes encoding modified target enzymes for certain herbicides that have a lower affinity for the herbicides than naturally produced endog-

enous enzymes, such as a modified glutamine synthetase as target for phosphinothricin as described in European patent publication 0,240,792 and a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate as described in European patent publication 0,218,571.

The second promoter, which controls the marker DNA, can also be selected and isolated in a well known manner so that the marker DNA is expressed either selectively in one or more specific tissues or specific cells or constitutively in the entire plant, as desired depending on the nature of the second RNA, protein or polypeptide encoded by the marker DNA. For example, if the marker DNA encodes an herbicide resistance, it may be useful to have the marker DNA expressed in all cells of the plant, using a strong constitutive second promoter such as a 35S promoter (Odell et al (1985) Nature 313, 810-812), a 35S'3 promoter (Hull and Howell (1987) Virology 86, 482-493), the promoter of the nopaline synthetase gene ("PNOS") of the Ti-plasmid (Herrera-Estrella (1983) Nature 303, 209-213) or the promoter of the octopine synthase gene ("POCS" [De Greve et al (1982) J. Mol. Appl. Genet. 1 (6), 499-511]). If the marker DNA encodes a protein conferring disease resistance, it may be useful to have the marker DNA selectively expressed in wound tissue by using, for example, a TR promoter such as the TR1' or TR2' promoter of the Ti-plasmid (Velten et al (1984) EMBO J. 3, 2723-2730). If the marker DNA encodes a herbicide resistance, it may be useful to have the marker DNA selectively expressed in green tissue by using, for example, the promoter of the gene encoding the small subunit of Rubisco (European patent publication 0,242,246). If the marker DNA encodes a pigment, it may be useful to have the marker DNA expressed in specific cells, such as petal cells, leaf cells or seed cells, preferably in the outside layer of the seed coat.

One can identify and isolate in a well known manner a tissue-specific second promoter for a plant to be rendered male-sterile and easily distinguishable from non-transformed plants by:

1. searching for an mRNA which is only present in the plant during the development of a certain tissue, such as its petals, leaves or seeds;
2. isolating this tissue-specific mRNA;
3. preparing a cDNA from this tissue-specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the tissue-specific mRNA; and then
5. identifying the portion of the plant genome that is upstream from the DNA coding for the tissue-specific mRNA and that contains the promoter for said DNA.

If more than one marker DNA is present in the foreign DNA sequence of this invention, all the marker DNAs can be under the control of a single second promoter, but preferably, each marker DNA is under the control of its own separate second promoter. More preferably, each marker DNA is under the control of its own second promoter and encodes a different second RNA, protein or polypeptide, providing different distinguishable characteristics to a transformed plant. In any event, the marker DNA(s) and second promoter(s) should be adjacent to each other and to the one or more male-sterility DNAs contained in the foreign DNA sequence of this invention and in any vector used to transform plant cells with the foreign DNA sequence.

It is generally preferred that the first RNA, protein or polypeptide, encoded by the male-sterility DNA, interfere significantly with the stamen cells' metabolism, functioning and/or development by acting in the cytoplasm or the nucleus of the stamen cells. However, when it is desired to have the first protein or polypeptide and/or of the second protein or polypeptide transported from the cytoplasm into chloroplasts or mitochondria of the cells of transformed plants, the foreign DNA sequence can further include an additional foreign DNA encoding a transit peptide. The additional DNA is between the male-sterility DNA and the first promoter if the first protein or polypeptide is to be so-transported and is between the marker DNA and the second promoter if the second protein or polypeptide is to be so-transported. By "transit peptide" is meant a polypeptide fragment which is normally associated with a chloroplast or mitochondrial protein or subunit of the protein and is produced in a cell as a precursor protein encoded by the nuclear DNA of the cell. The transit peptide is responsible for the translocation process of the nuclear-encoded chloroplast or mitochondrial protein or subunit into the chloroplast or the mitochondria, and during such a process, the transit peptide is separated or proteolytically removed from the chloroplast or mitochondrial protein or subunit. One or more of such additional DNA's can be provided in the foreign DNA sequence of this invention for transporting one or more first or second proteins or polypeptides as generally described in European patent publication 0,189,707 and in: Van den Broeck et al (1985) Nature 313, 358-363; Schatz (1987) Eur. J. of Bioch. 165, 1-6; and Boutry et al (1987) Nature 328, 340-342. An example of a suitable transit peptide for transport into chloroplasts is the transit peptide of the small subunit of the enzyme RUBP carboxylase (European patent publication 0,189,707) and an example of a transit peptide for transport into mitochondria is the transit peptide of the enzyme Mn-superoxide dismutase (see Example 16).

In the foreign DNA sequence of this invention, 3' transcription regulation signals can be selected among those which are capable of enabling correct transcription termination and polyadenylation of mRNA in plant cells. The transcription regulation signals can be the natural ones of the gene to be transcribed but can also be foreign or heterologous.

Examples of heterologous transcription regulation signals are those of the octopine synthase gene (Gielen et al (1984) EMBO J. 3, 835-845) and the T-DNA gene 7 (Velten and Schell (1985) Nucleic Acids Research ("NAR") 13, 6981-6998).

Also in accordance with this invention, plant cell cultures, such as anther cell cultures, containing the foreign DNA sequence of this invention in which the first promoter effects expression of the male-sterility DNA at a given stage of pollen development, more especially after meiosis, can be used to regenerate homozygous dominant male-sterile plants ("Efficient isolation of microspores and the production of microspore-derived embryos from Brassica napus", E. B. Swanson, M.P. Coumans, S.C. Wu, T.L. Barby and W.D. Beversdorf, Plant Cell Reports (1987) 6: 94-97).

Further in accordance with this invention, processes are provided for producing hybrid seeds which can be grown into hybrid plants. One process involves crossing a nuclear male-sterile plant including at least one marker DNA with a male-fertile plant without the marker DNA. Both male-sterile and male-fertile plants are planted in separate rows near to each other. Another process involves crossing a nuclear male-sterile plant including at least two different marker DNAs with a male-fertile plant including, in common, only one of the two different marker DNAs in a homozygous form. Both male-sterile and male-fertile parent plants can be grown in a substantially random population, increasing the chances of cross-pollination, without the need for precise planting patterns. The male-fertile parent plant can thereafter be easily removed from the population, using the distinctive trait encoded by the non-common marker DNA which is not possessed by the male-fertile parent plant. Preferably in this process, the non-common marker DNA in the male-sterile plant is under the control of a constitutive promoter and encodes a protein or polypeptide that renders the male-sterile plant resistant to a particular herbicide. The male-fertile plant can then be destroyed after cross-pollination, using the particular herbicide.

Plants, transformed with the male-sterility DNA, preferably with both the male-sterility DNA and the marker DNA encoding herbicide-resistance, stably integrated and transmissible throughout generations as dominant alleles in accordance with this invention, are alternatives to, and provide several advantages over, presently used cytoplasmic male-sterility systems for breeding and producing hybrid crops. Such advantages include:

1. For cross-pollinating crops, the breeding strategy is much simplified, because it is not necessary to introduce a restorer gene into the male-fertile parent line of the cross that will produce the commercially sold hybrid seed. Indeed, a heterozygous nuclear male-sterile parent line crossed with another male-fertile parent line for commercial seed production will produce 50% male-sterile hybrid offspring and 50% male-fertile hybrid offspring, as a result of which the commercial crop will produce enough pollen to guarantee full seed set and therefore normal yield. Examples for such crops are corn and oilseed rape.

2. For crops for which the seeds do not represent the economic harvest, the breeding strategy is also much simplified without the need of a restorer gene expressed in the male-fertile parent line. Indeed, for these crops it does not matter that 50% of the commercially sold hybrid seeds are male-sterile. Examples for these crops are sugarbeet and alfalfa.

3. The system allows production of nuclear male-sterile lines and maintainer lines from existing inbred lines in one operation, eliminating the need for backcrossing. This reduces the time lag between conception and commercialization of a hybrid by at least 6 to 8 generations. An example of a typical strategy for producing hybrid plants using as parent plant the plants having inserted and expressing the male-sterility DNA may consist of the following steps:

   1) making test hybrids by hand, by crossing inbred lines, and testing for combining ability and selected characteristics (2 years).
   2) making one parent line of each of the selected hybrids nuclear male-sterile by the process which is the object of this invention (1 year).
   3) multiplying the nuclear male sterile parent plant obtained from said process, hereinafter called "A^S", and its maintainer line, hereinafter called "A", and the pollinating male-fertile parent plant, hereinafter called "B", of the future commercial crop (3 years). During the same period, introducing the selected hybrids in official yield trials (3 years).
   4) producing and selling the approved hybrid seed (1 year).

4. Combined with a marker DNA encoding herbicide-resistance, such a nuclear male-sterility system allows production of 2-, 3- and 4- way hybrids in any combination required. It is believed to be sufficient to introduce the male-sterility DNA and adjacent thereto the marker DNA into the nuclear genome of one plant which will be used as one of the grandparent breeding lines for obtaining 2- or 3-way hybrids, and into the nuclear genome of two plants which will be used as the two grandparent lines for 4-way hybrids. Each breeding line can be maintained by the following two crosses given here by way of example, and whereby "SH" stands for the dominant alleles respectively of male-sterility (S) and herbicide resistance (H), and sh stands for the recessive alleles respectively of male fertility (s) and herbicide sensitivity (h):

a. SH/sh x sh/sh gives 50% SH/sh and 50% sh/sh offspring, and after spraying with the herbicide to which H confers resistance, 100% sterile seedlings are obtained.
b. sh/sh x sh/sh gives 100% fertile offspring.

5. It provides a protection for the owner of the marker DNA that has been integrated into the male-sterility system by making it more difficult for competitors to breed the marker DNA into their own breeding lines.

For illustrative purposes, two crop breeding schemes in accordance with this invention are given as follows:

Scheme 1: Breeding a plant containing adjacent male-sterility DNA and marker DNA encoding herbicide-resistance

1A) maintaining the male-sterility line A^{S}:

line $A^{SH/sh}$ x line $A^{sh/sh}$
giving
50% $A^{SH/sh}$ (phenotype: male-sterile, herbicide-resistant)
50% $A^{sh/sh}$ (phenotype: male-fertile, herbicide-susceptible)

1B) producing the hybrid seed crop:

a) planting seeds of $B^{sh/sh}$ (male plants) and the seeds obtained by the cross 1A) consisting of $A^{SH/sh}$ and $A^{sh/sh}$ ("female" plants) in separate rows.

b) eliminating the genotype $A^{sh/sh}$ by spraying the female rows with the herbicide.

c) cross-pollination occurring:

$A^{SH/sh}$ x $B^{sh/sh}$ and $B^{sh/sh}$ x $B^{sh/sh}$.
giving in the female rows:
50% $AB^{SH/sh}$ (phenotype: hybrid, male-sterile, herbicide-resistant)
50% $AB^{sh/sh}$ (phenotype: hybrid, male-fertile, herbicide-sensitive)
and in the male rows: 100% $B^{sh/sh}$.

d) eliminating the genotype $B^{sh/sh}$ occurring in the male rows by spraying with the herbicide or by mechanical means.

e) harvesting the hybrid seeds of the female rows wherein the cross-pollination of c) occurred. This is the commercially sold seed.

Scheme 2: Breeding a plant containing adjacent male-sterility DNA and two marker DNAs, each encoding a different herbicide-resistance (H1 and H2).

2A) maintaining the male-sterile line A^{S}:

$A^{SH1H2/sh1h2}$ x $A^{sh1h2/sh1h2}$
giving
50% $A^{SH1H2/sh1h2}$ (phenotype: male-sterile, resistant to both herbicides).
50% $A^{sh1h2/sh1h2}$ (phenotype: male-fertile, susceptible to both herbicides).

2B) maintaining pollination line B:

$B^{sh1H2/sh1H2}$ x $B^{sh1H2/sh1H2}$
giving
100% $B^{sh1H2/sh1H2}$ (phenotype: male-fertile, susceptible to herbicide 1 and resistant to herbicide 2).

2C) producing the hybrid seed crop:

a) planting the seeds obtained from 2A) and the seeds obtained from 2B) at random.

b) eliminating the genotype $A^{sh1h2/sh1h2}$ by spraying the field with herbicide 2.

c) cross-pollination occurring:

$A^{SH1H2/sh1h2} \times B^{sh1H2/sh1H2}$
giving
50% $AB^{SH1H2/sh1H2}$
50% $AB^{sh1h2/sh1H2}$
and
self-pollination occurring:
$B^{sh1H2/sh1H2} \times B^{sh1H2/sh1H2}$
giving
100% $B^{sh1H2/sh1H2}$

d) eliminating plants with genotype $B^{sh1H2/sh1H2}$ obtained from the parent line B, for which self-pollination occurred, by spraying the field with herbicide 1.

e) harvesting hybrid seeds of the remaining plants $A^{SH1H2/sh1H2}$ obtained by the cross-pollination of c).

The following Examples illustrate the invention. The figures referred to in the Examples are as follows:

Fig. 1 shows restriction maps of TA29 cDNA and its ClaI fragment in pTA29S3 of Example 1.
Fig. 2 shows the cDNA sequence of the PstI fragment of the TA29 gene of Example 2.
Fig. 3A shows the DNA sequence and amino acid sequence of the TA29 gene, from its ClaI site to its Hind III site. Above the sequences, the important restriction sites are indicated, and under the sequences is the amino acid sequence encoded by the ORF. Also indicated are: .

- from nucleotide ("nt") 1446 to 1452: TATA box (asterisks),
- at nt 1477: transcription initiation site of TA29 mRNA (asterisk),
- from nt 1514 to 1537: the 3' to 5' sequence of a synthetic oligomer as described in Example 2, and
- from nt 1940 to 2296 (between arrows); the aligned sequence of TA29 cDNA.

Fig. 3B shows the alignment of the TA13 cDNA (top line) and the TA29 cDNA (bottom line); as discussed in Example 4. Homologous nucleotides are indicated by vertical lines.
Fig. 3C shows the sequence of the TA26 cDNA, as discussed in Example 4; the ORF is underlined.
Fig. 4A shows schematically the construction of the vector pMB2 of Example 3.
Fig. 4B shows a map of the vector pMB3 of Example 3.
Fig. 5 shows a map of the vector pTTM3 of Example 5.
Fig. 6 shows a map of the vector pTTM4 of Example 7.
Fig. 7A shows a map of the vector pTTM6 of Example 9.
Fig. 7B shows a map of the vector PTTM6A- of Example 11.
Fig. 8 shows a map of the vector pTTM8 of Example 12.
Fig. 9A shows a map of the vector pTVEPl of Example 14.
Fig. 9B shows a map of the vector pTVEP2 of Example 14.
Fig. 10A shows a map of the vector pTVEP63 of Example 16.
Fig. 10B shows a map of the vector pTVEP62 of Example 16.
Fig. 11 shows a photograph of flowers of normal tobacco plants compared with flowers of tobacco plants transformed with the male-sterility DNA of Example 9.
Fig.12 shows a photograph of a transverse cutting of the anther of a normal tobacco plant compared with the anther of a tobacco plant transformed with the male-sterility DNA of Example 9 (enhancement: x 250).

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA were carried out by the standardized procedures described in Maniatis et al, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory (1982). The following plasmids and vectors, used in the Examples, have been deposited in the Deutsche Sammlung Für Mikroorganismen und Zellculturen ("DSM"), Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany under the provisions of the Budapest Treaty:

Plasmid or vector	DSM Accession No.	Date
pMB3	4470	21 Mar. 1988
pGSC1600	4467	21 Mar. 1988
pGSC1700	4469	21 Mar. 1988
pGV2260	2799	Dec. 1983
PGSC1701A	4286	22 Oct. 1987
pTTM4	4471	21 Mar. 1988
pTTM6	4468	21 Mar. 1988

Example 1 - Subcloning of an anther-specific gene (the "TA29 gene")

From Professor Robert Goldberg of the University of California, Los Angeles (UCLA) were obtained: a Nicotiana tabacum anther-specific cDNA ("TA29 cDNA") cloned as a PstI fragment in pBR329 (Covarrubias and Bolivar (1982) Gene 17, 79) by GC tailing; and the corresponding genomic clone ("lambda TA29") that was isolated from a N. tabacum "Samsun" genomic library using TA29 cDNA as a probe and that was inserted in the EcoRI site of the lambda phage vector cH32 (Loenen and Blattner (1983) Gene 26, 171). The TA29 cDNA was 365 base pairs long (±0.4 kb) and hybridized to a tapetum-specific mRNA of 1,100 nucleotides which accounts for 0.24% of the poly A^+ mRNA from anthers of the N. tabacum. As shown in Fig. 1, lambda TA29 contains two EcoRI fragments, the total insert measuring 13.2 kb.

An internal 7.5 kb ClaI fragment as shown in Fig. 1, containing the TA29 gene, was subcloned from lambda TA29 in pLK31 (Botterman and Zabeau (1987) DNA 6, 6) which produced a plasmid named "pTA29S3". Nitrocellulose bound fragments of lambda TA29, digested with the combination of EcoRI/ClaI/HindIII/HindIII-EcoRI and the combination of ClaI-EcoRI and hybridized against TA29 cDNA, indicated the presence of sequences homologous to TA29 cDNA.

Example 2 - Nucleotide sequence determination of TA29 cDNA and its homologous sequence from pTA29S3; mapping of TA29 gene and its promoter.

The PstI insert of TA29 cDNA in pBR329 was completely sequenced (Maxam and Gilbert (1977) Proc. Natl. Acad. Sci. USA ("PNAS") 74, 560). The cDNA sequence is shown in Fig. 2. It reveals the presence of one open reading frame over the entire cDNA sequence (as indicated).

Then, the sequence of the ClaI insert in pTA29S3 was determined from the ClaI site to the HindIII site (3261 base pairs apart). Comparison of the TA29 cDNA sequence and the pTA29S3 sequence revealed the presence of a sequence in the pTA29S3 which was completely homologous with the TA29 cDNA sequence.

Figure 3 shows the sequence of the TA29 gene in pTA29S3. The sequence in pTA29S3 that is identical to the TA29 cDNA sequence is between the arrows in Fig. 3. A putative open reading frame is revealed by the corresponding amino acid sequence in Fig. 3. This indicates that the TA29 gene encodes a protein of 321 amino acid residues and that there are no introns present in the coding region. The length of the open reading frame of 964 (+ leader) nucleotides matches the size of a transcript present in tobacco anther mRNA prepared from anthers isolated from young (12-20 mm long) tobacco flower buds and absent in the mRNA isolated from leaf and older flowers (when the buds are opened and petals have appeared). The size of this mRNA is approximately 1100 nucleotides.

There are two ATG codons, one at nucleotide ("nt") 1527 and the other at nt 1560, which could serve as initiation codon for the open reading frame, 33 nucleotides apart. There is a consensus sequence TATA at nt 1446 present 81 nucleotides 5' upstream of the first ATG codon (indicated by asterisks in Fig. 3). To confirm that this "TATA" box is part of the promoter of the TA29 gene, the 5' end of the TA29 mRNA was determined. This was done by primer extension (Mc Knight et al (1981) Cell 25, 385). For this purpose, an oligomer of 24 nucleotides, having the sequence: 5' GGA GCT ACC ATT TTA GCT AAT TTC 3', was used as it is complementary to the TA29 gene from nt 1514 to nt 1537 as shown in Fig. 3.

This oligonucleotide was ^{32}P labeled by kination at the 5' end. After being hybridized with anther mRNA, the oligonucleotide was extended by reverse transcriptase. The resulting extended oligonucleotide was analyzed on a sequencing gel, next to a sequencing ladder, to determine its exact size. The fragment was shown to be 61 nucleotides long. This indicates that transcription initiation of the TA29 mRNA occurred at nt 1477 (indicated by asterisk in Fig. 3). Hence, the TA29 gene has a TATA box located 31 nucleotides upstream of the transcription initiation site. The mRNA contains a 51 nucleotide-long leader sequence from nt 1477 to nt 1527, a coding region of 964 nucleotides from nt 1527 to nt 2491, and a 3' non coding region of approximately 100 nucleotides from nt 2492 to nt 2590. As is the case in approximately 92% of presently characterized plant genes (Jashi (1987) Nucleic Acids Research ("NAR") 15 (16),

6643), it is believed that the first AUG codon of the mRNA is used to initiate translation. The TA29 promoter thus appears to be located between the ClaI restriction site and nt 1477.

Example 3 - Construction of a promoter cassette ("PTA29") derived from the TA29 gene

To construct chimaeric DNA sequences containing the 5' regulatory sequences, including the promoter, of the TA29 gene in the same transcriptional unit as, and controlling, a first heterologous male-sterility DNA, a cassette was constructed as shown in Fig. 4 by subcloning a 2.5 kb ClaI/AccI fragment from pTA29S3 into the polylinker AccI site of the pMAC 5-8 vector system (European patent application 87/402348.4 which is the priority document of European patent publication 0,319,353). This produced a vector named "pMB2", shown in Fig. 4, which could be used to isolate single strand DNA for use in site directed mutagenesis.

Then, the sequence surrounding the first ATG codon AAAATGGTA was modified to ACCATGGTA by substituting two adenine residues for cytosine residues. This mutation created the sequence CCATGG which is the recognition site for the restriction enzyme NcoI. This site directed mutagenesis in pMB2 was performed using a synthetic oligonucleotide of 24 nucleotides with the following sequence:

3'GTT TAA TCG ATG GTA CCA TCG AGG 5'

The resulting plasmid, containing the newly created NcoI site, was named "pMB3" and is shown in Fig. 4 B . The precise nucleotide sequence spanning the NcoI site was determined in order to confirm that it only differed from the 5' sequence of the TA29 gene by the AA -- CC substitution, creating the NcoI site. The 1507 nucleotide long fragment ClaI -- NcoI was named "PTA29".

Example 4 - Identification of cDNA clones obtained from other stamen-specific mRNAs

To demonstrate that other anther-specific mRNAs could be identified and then used to isolate cDNA clones with analogous properties to the TA29 gene, two other N. tabacum anther-specific cDNAs ("TA13 cDNA" and "TA26 cDNA") were obtained from Professor Goldberg of UCLA.

TA13 cDNA is a clone of 1100 bp which hybridized to two mRNA species of about 1100 and 1200 nucleotides, respectively, which are specific for tapetum cells and are abundant at a very early stage of anther development. TA13 cDNA was sequenced, using the procedure of Example 2, and then compared with the sequence of TA29 cDNA as shown in Fig. 3B. This sequence comparison reveals that TA13 cDNA and TA29 cDNA share 92% homology, and the ORF is very rich in glycine content.

TA26 cDNA was cloned as a PstI insert into pBR329 by poly-G/C tailing. It is a clone of 519 bp which hybridized to one tobacco mRNA species of 580 nucleotides, which mRNA is specific for tapetum cells and abundant at a certain stage of anther development. The entire TA26 cDNa was sequenced, using the procedure of Example 2, and when compared with the sequence of TA29 cDNA, revealed no homology. The sequence of TA26 cDNA is given in Fig. 3C.

Example 5 - Construction of a chimaeric DNA sequence of PTA29 and a glucuronidase gene

A plasmid named "pTTM3", shown in Fig. 5, was constructed by assembling the following well known DNA fragments with PTA29:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1600;
2. a chimeric sequence containing the promoter cassette PTA29 from Example 3, fused in frame with a pMB3 NcoI/EcoRI fragment containing an E. coli gene encoding beta-glucuronidase ("GUS" [Jefferson et al (1986) PNAS 83, 8447; Jefferson et al (1987) EMBO J. 6, 3901]) and the 3' end signals of an octopine-synthase gene ("OCS" [Dhaese et al (1983) EMBO J. 2, 419]);
3. a chimaeric sequence containing an Arabidopsis SSU promotor ("PSSU" or "PSSUARA") , a herbicide resistance gene sfr (European patent publication 0,242,246) and the 3' end signals of a T-DNA gene 7 (Velten and Schell (1985) NAR 13, 6981); and
4. a chimaeric sequence containing the EcoRI/SacI fragment from pGSFR401 which contains a nopaline-synthase promoter ("PNOS"), a neo gene encoding kanamycin resistance and the 3' end signals of an octopine synthase gene (European patent publication 0,242,246 wherein pGSFR401 is called "pGSR4").

pTTM3 is a T-DNA vector containing, within the T-DNA border sequences, two chimaeric sequences: PSSU-sfr in which the sfr is a marker DNA (European patent publication 0,242,246) under the control of PSSU as a second promoter;

and PTA29-GUS in which GUS is a reporter gene whose expression in plants and plant cells under the control of the TA29 promoter can easily be localized and quantified.

Example 6 - Introduction of the chimaeric DNA sequence of Example 5 into tobacco

A recombinant Agrobacterium strain was constructed by mobilizing pTTM3 (from Example 5) from E. coli into Agrobacterium C58C1 Rif^R containing pGV2260 (De Blaere et al (1985) NAR 13, 4777). Mobilization was carried out using E. coli HB101 containing pRK2013 (Figurski et al (1979) PNAS 76, 1648) as a helper as described in European patent publication 0,116,718. The resulting Agrobacterium strain contained a hybrid Ti-plasmid comprising pGV2260 and pTTM3.

This strain was used to tramform tobacco leaf discs (N. tabacum Petite Havane SR1) using standard procedures as described, for example, in European patent publication 0,242,246. Transformed calli and shoots were selected using 5 mg/l of the herbicide phosphinothricin in the medium (De Block et al (1987) EMBo J. 6, 2513). No beta-glucuronidase enzyme activity detected in the transformed herbicide-resistant calli and shoots.

Then, the transformed shoots were rooted, transferred to soil in the greenhouse and grown until they flowered. The flowers were examined, and only the tapetum cells in the anthers of the stamen were found to contain beta-glucuronidase activity. This shows that the TA29 promoter is capable of directing expression of a heterologous gene, like the beta-glucuronidase gene, selectively in tapetum cells of the plants.

Example 7 - Construction of a chimaeric DNA sequence of PTA29 and a gene 4

A plasmid named "pTTM4", shown in Fig. 6, was constructed by assembling the following well known DNA fragments with PTA29.

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 (Cornelissen and Vandewiele (1989) NAR 17 (1), 19-29);
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter controlling expression of herbicide-resistance gene sfr and the 3' end of a T-DNA gene 7;
3. the chimaeric sequences (no. 4) of Example 5, containing the PNOS promoter controlling expression of the neo gene and the 3' end of the octopine synthase gene; and
4. a chimaeric sequence containing the PTA29 promotor cassette from Example 3, fused in frame with an Agrobacterium T-DNA gene 4 that encodes isopentenyl transferase (Akiyoahi et al (1984) PNAS 76, 5994; Barry et al (1984) PNAS 81, 4776) containing its own 3' end transcription regulation signals.

pTTM4 is a binary type T-DNA vector containing, within the T-DNA border sequences, the following chimaeric sequences: PSSU-sfr and PNOS-neo in which the sfr and neo genes are marker DNAs that encode dominant selectable markers for plants and that are under the control of respectively PSSU and PNOS as second promoters; and PTA29-gene 4 in which gene 4 is a male-sterility DNA that is under the control of PTA29 as a first promoter and encodes the enzyme isopentenyl transferase which will cause the enhanced production of cytokinin. Enhanced cytokinin production in tapetum cells, under the control of the TA29 promoter, will disturb the metabolism and organogenesis of the tapetum cells.

Example 8 - Introduction of the chimaeric DNA sequence of Example 7 into tobacco

As described in Example 6, pTMM4 (from Example 7) was introduced with mobilization from E. coli into Agrobacterium C58C1 Rif^R. The resulting Agrobacterium strain contained a binary type Ti-plasmid system comprising pGV2260 and pTTM4.

As also described in Example 6, this strain was used to transform tobacco leaf discs, and transformed calli and shoots were selected using 5 mg/l of phosphinothricin. Transformed herbicide-resistant shoots were rooted, which shows that gene 4 was not yet being expressed in the transformed plants.

The plants were then transferred to soil in the greenhouse and grown until they flower. The flowers are examined, and no functional tapetum cells are found in their anthers of their stamen. This shows that the TA29 promoter is capable of directing expression of the heterologous gene 4 selectively in tapetum cells of the plants.

Example 9 - Construction of a chimaeric DNA sequence of PTA29 and a RNAse T1 gene

A plasmid named "pTTM6", shown in Fig. 7A, was constructed by assembling the following well known DNA fragments with PTA29:

1. a vector fragment, including T-DNA border sequences, from pGSC1600;
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promotor, the herbicide resistance gene sfr

and the 3' end of the T-DNA gene 7; and

3. a chimaeric sequence, containing the pTA29 promoter cassette from Example 3, fused in frame with a synthetic gene encoding RNase T1 from A. orhyzae, (Quaas et al, "Biophosphates and their Analogues-Synthese, Structure, Metabolism and Activity" (1987) Elsevier Science Publisher B.V., Amsterdam; Quaas et al (1988) Eur. J. Biochem, 173, 617-622.) and the 3' end signals of a nopaline synthase ("NOS") gene (An et al (1985) ENBO J. 4 (2), 277).

pTTM6 is a T-DNA vector containing, within the T-DNA border sequences, two chimaeric sequences; PSSU-sfr which is a marker DNA under the control of PSSU as a second promoter; and PTA29-RNase T1 gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Expression in tapetum cells of the male-sterility DNA under the control of the TA29 promoter will produce RNase T1 that will be lethal for the cells, since the RNase T1 will degrade the RNA molecules which are indispensable for these cells' metabolism.

Example 10 - Introduction of the chimaeric DNA sequence of Example 9 into tobacco

As described in Example 6, a recombinant Agrobacterium strain was constructed by mobilization of pTTM6 (from Example 9) from E. coli into Agrobacterium C58C1 Rif^R. The resulting Agrobacterium strain, harboring a cointegrated Ti-plasmid comprised of pGV2260 and pTTM6, was used for transforming tobacco leaf discs. Transformed calli and shoots were selected using 5 mg/l phosphinothricin. That the RNase T1 gene was not expressed in the transformed herbicide-resistant calli and shoots was shown by their growth.

The transformed shoots were rooted, transferred to soil in the greenhouse and grown until they flowered. The transformed tobacco plants developed normal flowers except for their anthers. The anthers, although of normal shape, dehisced later in time, compared to the anthers of non-transformed tobacco plants (see Fig. 11). Upon dehiscense, either little or no pollen was released from the transformed plants, and the pollen grains formed by the transformed plants, were about 50 to 100 times smaller in volume than normal- pollen grains and were irregularly shaped. Moreover, most of the pollen grains from transformed plants failed to germinate, and the germination efficiency of pollen from transformed plants was about 0 to 2% of the germination efficiency of normal pollen grains. Furthermore, the transformed plants did not produce any seeds by self-pollination -- neither by natural self-pollination nor by hand-provoked self-pollination.

Microscopic evaluation, by thin layer cross section, of a transformed plant showed that no normal tapetum layer was formed and that the pollen sack remained empty (see Fig. 12). This shows that the TA29 promoter is capable of directing expression of the heterologous RNase T1 gene selectively in tapetum cells of the transformed plants, and that the RNase TI is capable of sufficiently disturbing the functioning of the tapetum cells, so as to render the plants male-sterile.

Example 11 - Introduction of a derivative of the chimaeric DNA sequence of Example 9 into oilseed rape

A recombinant Agrobacterium strain was constructed by mobilization of $pTTM6A^-$ from E. coli into Agrobacterium C58 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204, 383-396). pMP90 provides vir and trans functions and does not carry a gene encoding ampicillin resistance. As shown in Fig. 7B, $pTTM6A^-$ is a derivative of PTTM6 (from Example 9), in which the β-lactamase gene encoding ampicillin resistance has been inactivated by insertion of a DNA sequence into the ScaI site of the β-lactamase gene.

The resulting Agrobacterium strain (named "A3144"), harboring pMP90 and $pTTM6A^-$, was used for the transformation of Brassica napus according to the procedure of Lloyd et al (1986) Science 234, 464-466 and Klimaszewska et al (1985) Plant Cell Tissue Organ Culture 4, 183-197. Carbenicillin was used to kill A3144 after co-cultivation occurred. Transformed calli were selected on 5 mg/l phosphinothricin and 100 ug/ml kanamycin, and resistant calli were regenerated into plants. After induction of shoots and roots, the transformants were transferred to the greenhouse and grown until they flower. The flowers are examined, and they exhibit essentially the same phenotype as was observed for the transformed tobacco plants described in Example 10. This shows that the TA29 promoter is capable of directing the expression of the heterologous RNase T1 gene selectively in tapetum cells of plants other than tobacco, so as to render such other plants male-sterile.

Example 12 - Construction of a chimaeric DNA sequence of PTA29 and a Bamase gene

A plasmid named "pTTM8" shown in Fig. 8, was constructed by assembling the following well known fragments with PTA29:

1. a vector fragment, including T-DNA border sequences derived from pGSC1700 (Cornelissen and Vandewiele (1989) NAR 17 (1) 19-29) and in which the β-lactamase gene (1' of Fig. 8) has been inactivated by insertion of a

DNA sequence into its ScaI site;
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3' end of T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene, and the 3' end of the octopine synthase gene; and
4. a chimaeric sequence, containing the PTA29 promoter cassette from Example 3, fused in frame with the Barnase gene from Bacillus amyloliquefaciens (Hartley and Rogerson (1972) Preparative Biochemistry 2, (3), 243-250) and the 3' end of the nopaline synthase gene of Example 9.

pTTM8 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are markers DNAs with respectively PSSU and PNOS as second promoters; and PTA29-Barnase gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Expression in tapetum cells of the male-sterility DNA under the control of the TA29 promoter will produce Barnase selectively in the tapetum cells so that Barnase will interfere with the metabolism of these cells.

Example 13 - Introduction of the chimaeric DNA sequence of Example 12 into tobacco and oilseed rape

As described in Example 11, a recombinant Agrobacterium strain was constructed by mobilizing pTTM8 (from Example 12) from E. coli into Agrobacterium C58C1 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204, 383-396). The resulting strain (named "A3135"), harboring pMP90 and pTTM8, is used for tobacco leaf disc transformation and for oilseed rape transformation. Transformed calli and shoots are selected using 5mg/l phosphinothricin and 100 ug/ml kanamycin. That the Barnase gene is not expressed in the transformed herbicide-resistant calli and shoots is shown by their growth.

The transformed shoots are rooted, transferred to soil in the greenhouse and grown until they flower. The flowers of both the tobacco and oilseed rape are examined, and a phenotype is observed for the transformed plants that is essentially the same as the phenotype of the transformed tobacco plants described in Example 10. This shows that the TA29 promoter is capable of directing expression of the heterologous Barnase gene selectively in tapetum cells of the plants, thereby rendering the plants male-sterile.

Example 14 - Construction of a chimaeric DNA sequence of pTA29 and a gene encoding papain

A plasmid named "pTVEPI", shown in Fig. 9A, is constructed by assembling the following well known fragments with PTA29:

1. a vector fragment, including T-DNA border sequences derived from pGSC1700 and in which the β-lactamase gene (1' of Fig. 9A) has been inactivated by insertion of a DNA sequence into its ScaI site;
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide resistance gene sfr and the 3' end of T-DNA gene 7.
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the 3' end of the octopine synthase gene; and
4. a chimaeric sequence, containing the PTA29 promoter cassette from Example 3, fused in frame with:

   a) a papain gene from Carica papaya fruit, encoding the papain zymogen which is a plant endopeptidase (Cohen et al (1986) Gene 48, 219-227) capable of attacking peptide, as well as ester, bonds; the following modifications are made in the DNA sequence of Cohen et al (1986) using site directed mutagenesis as described in Example 3:

      i. the nucleotide A, position-1 upstream of the first ATG codon, is mutated into nucleotide C in order to obtain a suitable NcoI cloning site; and
      ii. the GAA codons encoding glutamate at positions 47, 118, 135, respectively, are mutated into CAA codons encoding glutamine; and

   b) the 3' end of the nopaline synthase gene of Example 9.

pTVEP1 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs encoding dominant selectable markers for plant transformations, under the control of respectively PSSU and PNOS as second promoters; and PTA29-Papain gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Expression in tapetum cells of the male-sterility DNA under

the control of the TA29 promoter will produce an endopeptidase (the papain zymogen) that will cleave proteins in the tapetum cells, thus leading to the death of these cells.

A plasmid named "pTVEP2", shown in Fig. 9B, is also constructed by assembling the following well known fragments with PTA29:

1. a vector fragment, including T-DNA border sequences derived from pGSC1700 and in which the β-lactamase gene (1' of Fig. 9B) has been inactivated by insertion of a DNA sequence into the ScaI site;
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide resistance gene sfr and the 3' end of T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene, and the 3' end of the octopine synthase gene; and
4. a chimaeric sequence, containing the PTA29 promoter cassette of Example 3, fused in frame with:

    a) a papain gene from Carica papaya fruit, encoding the active protein of the papain zymogen; the following modifications are made in the DNA sequence of Cohen et al (1986), using site directed mutagenesis as described in Example 3:

        i. the AAT codon encoding Asn, upstream of the first Ile residue of the active protein, is mutated into a GAT codon, which provides a suitable EcoRV cloning site (GAT ATC). The EcoRV engineered site is fused directly to the pTA29 cassette in order to obtain a direct in frame fusion of the promoter with the sequence encoding the active protein of the papain zymogen; and
        ii. the GAA codons encoding glutamate at positions 47, 118, 135 respectively, are mutated into CAA codons encoding glutamine; and .

    b) the 3' end of the nopaline synthase gene of Example 9.

pTVEP2, like pTVEP1, is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric genes: PSSU-sfr and PNOS-neo encoding dominant selectable markers for plant transformations; and PTA29-Papain gene which encodes an endopeptidase that will cleave proteins in the tapetum cells, thus leading to the death of these cells.

Example 15 - Introduction of the chimaeric DNA sequences of Example 14 into tobacco and oilseed rape

As described in Example 11, pTVEP1 and pTVEP2, are each mobilized from E. coli into separate Agrobacterium C58C1 Rif^R carrying pMP90.

The resulting strains, harboring pMP90 with pTVEP1 and pMP90 with pTVEP2, are used to transform tobacco and oilseed rape following the procedures of Examples 11 and 13. That the papain genes are not expressed in transformed herbicide-and kanamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The flowers of both the tobacco and oilseed rape are examined, and phenotypes are observed for the transformed plants that are essentially the same as the phenotype of the transformed tobacco plants described in Example 10. This shows that the TA29 promoter is capable of directing expression of the heterologous papain genes in pTVEP1 and pTVEP2 selectively in tapetum cells of the plants, thereby rendering the plants male-sterile.

Example 16 - Construction of a chimaeric DNA sequence of pTA29 and a gene encoding EcoRI

A plasmid named "pTVE63", shown in Fig.10A, was constructed by assembling the following well known fragments with PTA29:

1. a vector fragment, including T-DNA border sequences derived from pGsC1701A2 (European patent publication 0,270,822);
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3' end of T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the 3' end of the octopine synthase gene;
4. a chimaeric sequence, containing the pTA29 promoter cassette of Example 3, fused in frame with:

    a) a gene encoding the EcoRI restriction endonuclease from an E. coli (Green et al (1981) J. Biol. Chem. 256,

2143-2153; Botterman and Zabeau (1985) Gene 37, 229-239) and capable of recognizing and cleaving the target sequence GAATTC on a double stranded DNA; the following modifications were made in the DNA sequence of Green et al (1981) using site directed mutagenesis as described in Example 3:

i. the nucleotides of the ATG initiation codon were replaced by ATGCA, creating a NsiI site at the initiation codon and yielding the following nucleotide sequences:

ATGCA,TCT,AAT...;

and

ii. the HindII-HindIII fragment of the EcoRI gene cloned in pEcoR12 (Botterman and Zabeau, 1985) was cloned into the pMAC5-8 site directed mutagenesis vector; and

b) the 3' end of the nopaline synthase gene of Example 9; and

5. a gene encoding an EcoRI methylase under the control of its natural promoter (Botterman and Zabeau (1985) Gene 37, 229-239) which is capable of inhibiting the activity of EcoRI in E. coli or Agrobacterium, in order to overcome potential leaky expression of the EcoRI gene in microorganisms.

pTVE63 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs under the control of respectively PSSU and PNOS as second promoters; and PTA29-EcoRI gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Expression of the male-sterility DNA under the control of the TA29 promoter in tapetum cells will produce the EcoRI restriction endonuclease which will cleave double stranded DNA at the GAATTC sites (see for review of type II restriction modification systems: Wilson (1988) TIG 4 (11), 314-318) of the tapetum cells, thus leading to the death of these cells.

A plasmid named pTVE62, shown in Fig.10B, was also constructed by assembling the following well known fragments with PTA29:

1. a vector fragment, including T-DNA border sequences derived from pGSC1701A2;
2. the chimaeric sequence (no. 3) of Example 5, containing the pSSU promoter, the herbicide-resistance gene sfr and the 3' end of T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the neo 3' end of the octopine synthase gene;
4. a chimaeric sequence, containing the pTA29 promoter cassette of Example 3, fused in frame with a gene fragment encoding the transit peptide of the Mn-superoxide dismutase ("Mn-SOD") which is a NcoI-PstI fragment of a HpaI-HindIII fragment from pSOD1 (Bowler et al (1989) Embo J. 8, 31-38); the following modifications were made in the DNA sequence of Bowler et al using site directed mutagenesis as described in Example 3:

i. the AA nucleotides located upstream at position -2 and -1 of the ATG initiation codon were changed to CC nucleotides creating a NcoI site at the initiation codon and yielding the following nucleotide sequences:

- CCATGGCACTAC

ii. the T,TCG,CTC, nucleotides located immediately downstream of the processing site of the transit peptide were changed to C,TGC,AGC, creating a PstI site behind the processing site and yielding the the following nucleotide sequences:

```
                    | L   Q   T   F   S   L
CTC,CGC,GGC,        | TTG,CAG,ACC,TTT,TCG,CTC
CTC,CGC,GGC,        | TTG,CAG,ACC,TTC,TGC,AGC...
                    |                 PstI
```

in which the arrow indicated the processing site of the transit peptide sequence and the upper line the ami-

noacid sequence corresponding with the Mn-SOD coding sequence; the NcoI-PstI: fragment was also fused in frame with a gene encoding the EcoRI restriction endonuclease from E. coli (Greene et al (1981) J. Biol. Chem. 256, 2143-2153; Botterman and Zabeau (1985) Gene 37, 229-239) and capable of recognition and cleavage of the target sequence GAATTC on a double stranded DNA, as found in pTVE63: and

b) the 3' end of the nopaline synthase gene of Example 9: and

5. a gene encoding the EcoRI methylase under the control of its natural promoter (Botterman and Zabeau. 1985) which is capable of inhibiting the activity of EcoRI in E. coli or Agrobacterium, in order to overcome potential leaky expression of the EcoRI gene in microorganisms, this gene being inserted into the vector fragment outside the border sequences.

pTVE62 is a binary type T-DNA vector containing, within the border sequences, three chimeric sequences: PSSU-sfr and PNOS-NPTII which are marker DNAs under the control of respectively PSSU and PNOS as second promoters; and pTA29-transit peptide-EcoRI endonuclease gene which is a male-sterility DNA having PTA29 as a first promotor and a transit peptide-encoding sequence between them. Expression of the male-sterility DNA under the control of the TA29 promoter in tapetum cells will produce a restriction endonuclease which will be targeted into the mitochondria of the tapetum cells and cleave the double stranded DNA at the GAATTC sites in such cells. This will lead to the death of these cells.

Example 17 - Introduction of the chimaeric DNA sequences of Example 16 into tobacco and oilseed rape

As described in Examples 11 and 15, pTVE62 and pTVE63, were mobilized from E. coli into Agrobacterium C58C1 Rif^R carrying pMP90. The resulting strains, harboring pTVE62 with pMP90 (or pTVE63 with pMP90), were used to transform tobacco and are used to transform oilseed rape following the procedures described in Examples 11 and 13. That the EcoRI endonuclease genes were not expressed in transformed herbicide- and kanamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The flowers of both the tobacco and oilseed rape are examined, and phenotypes are observed for the transformed plants that are essentially the same as of the tramformed tobacco plants described in Example 10. This shows that the TA29 promoter is capable of directing expression of the heterologous EcoRI endonuclease gene selectively in the tapetum cells of the plants tramformed with pTVE62 and pTVE63, thereby rendering the plants male-sterile.

Needless to say, this invention is not limited to the transformation of any specific plant(s). The invention relates to any plant, the nuclear genome of which can be transformed with a male-sterility DNA under the control of a first promoter that can direct expression of the male-sterility DNA selectively in the plant's stamen cells, whereby the plant can be both self-pollinated and cross-pollinated. For example, this invention relates to plants such as potato, tomato, oilseed rape, alfalfa, sunflower, cotton, celery, onion, corn, soybean, tobacco, brassica vegetables and sugarbeet,

Also, this invention is not limited to the specific plasmids and vectors described in the foregoing Examples, but rather encompasses any plasmids and vectors containing the male-sterility DNA under the control of the first promoter.

Furthermore, this invention is not limited to the specific promoters described in the foregoing Examples, such as the TA29 promoter, but rather encompasses any DNA sequence encoding a promoter capable of directing expression of the male-sterility DNA selectively in stamen cells. In this regard, this invention encompasses the DNA sequence of the TA29 promoter of Fig. 3A, as well as any equivalent DNA sequences. Indeed, it is believed that the DNA sequences of the TA29 promoter can be modified by: 1) replacing some codons with others that code either for the same amino acids or for other amino acids; and/or 2) deleting or adding some codons; provided that such modifications do not substantially alter the properties of the encoded promoter for controlling tapetum-specific expression of a male-sterility.

In addition, this invention is not limited to the specific male-sterility DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding a first RNA, protein or polypeptide which disturbs significantly the metabolism functioning and/or development of a stamen cell in which it is produced, under the control of the first promoter.

Also, this invention is not limited to the specific marker DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding a second RNA, protein or polypeptide which confers on at least a specific plant tissue or specific plant cells, in which such DNA sequence is expressed, a distinctive trait compared to such a specific plant tissue or specific plant cells in which such DNA sequence is not expressed.

Claims

1. A male-sterile plant containing a foreign DNA incorporated in the nuclear genome of all of its cells, wherein said

foreign DNA comprises:

(a) a male-sterility DNA encoding a first RNA, protein or polypeptide, capable when produced in stamen cells of said plant, of killing or disabling them to prevent the production of fertile male gametes;
(b) a first promoter which directs gene expression selectively in stamen cells of said plant, said male-sterility DNA being in the same transcriptional unit as, and under the control of said first promoter,

provided that, if said first promoter is a promoter which directs expression of said male-sterility DNA selectively in microspore and/or pollen cells, the nuclear genome of the cells of said plant is homozygous.

2. The plant of claim 1, wherein said first promoter directs expression of said male-sterility DNA in anther cells.

3. The plant of claim 2 wherein said first promoter directs expression of said male-sterility DNA in tapetum cells or anther epidermal cells, of said plant.

4. The plant of any one of claims 1 to 3, wherein said first promoter is the promoter of the TA29 gene of Fig. 3A.

5. The plant of any one of claims 1 to 3, wherein said first promoter is the promoter of the endogenous tobacco TA26 gene encoding a mRNA from which a cDNA comprising the nucleotide sequence of Fig. 3C can be prepared, the promoter of the endogenous tobacco TA13 gene encoding a mRNA from which a cDNA comprising the nucleotide sequence of Fig. 3B can be prepared, or a promoter of an endogenous gene of a plant coding for a tapetum-specific mRNA hybridizable to said TA29 gene, said TA26 gene or said TA13 gene.

6. The plant of claim 1 which is a homozygous plant and in which said first promoter directs expression of said male-sterility DNA selectively in pollen cells of said homozygous plant.

7. The plant of any one of claims 1 to 6, wherein said male-sterility DNA encodes: an RNase, particularly RNase T1.

8. The plant of any one of claims 1 to 6, wherein said male-sterility DNA encodes barnase

9. The plant of any one of claims 1 to 6, wherein said male-sterility DNA encodes: a DNase, especially an endonuclease, particularly EcoRI; a protease, especially a papain, particularly papain Zymogen or papain active protein; a glucanase; a lipase, particularly phospholipase A2; a lipid peroxidase; a cell wall inhibitor; a bacterial toxin, particularly the A-fragment of diptheria toxin; or a ribozyme, particularly the ribozyme against mRNA encoded by the TA29 gene of Fig. 3A, against mRNA of the TA26 gene from which a cDNA comprising the sequence of Fig. 3C can be prepared, or against mRNA from which a cDNA comprising the sequence of Fig. 3B can be prepared; or is an antisense DNA which encodes an RNA that is complementary to mRNA of said TA29 gene, said TA26 gene or said TA13 gene.

10. The plant of any one of claims 1 to 6, wherein said male-sterility DNA encodes an enzyme which catalyzes the synthesis of a phytohormone, particularly the enzyme encoded by gene 4 of *Agrobacterium* T-DNA, or an enzyme encoded by gene 1 and/or gene 2 of *Agrobacterium* T-DNA.

11. The plant of any one of claims 1 to 10, wherein said foreign DNA further comprises :
(e) a first DNA encoding a transit peptide capable of transporting said first protein or polypeptide into a chloroplast or mitochondria of said stamen cells; said first DNA being in the same transcriptional unit as said male-sterility DNA and said first promoter and between said male-sterility DNA and said first promoter.

12. A plant of any one of claims 1 to 11 which is selected from the group of corn, potato, tomato, oilseed rape, alfalfa, sunflower, cotton, celery, onion, clover, soybean, tobacco, brassica vegetables or sugarbeet.

13. The plant of any one of claims 1 to 12, wherein said foreign DNA also comprises :

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or in at least specific cells of said plant, renders said plant easily separable from other plants which do not contain said second RNA, protein or polypeptide in said specific tissue or specific cells; and
(d) a second promoter capable of directing expression of said marker DNA at least in said specific tissue or specific cells; said marker DNA being in the same transcriptional unit as, and under the control of, said second

promoter.

14. The plant of claim 13 wherein said marker DNA encodes a protein inhibiting or neutralizing the action of a herbicide.

15. The plant of claim 14 in which said marker DNA is an herbicide resistance gene, particularly a gene conferring resistance to a glutamine synthetase inhibitor, such as phosphinothricin.

16. The plant of claim 15 in which said marker DNA is a sfr or sfrv gene.

17. The plant of claim 14 wherein said marker DNA encodes a modified target enzyme for an herbicide having lower affinity for the herbicide, particularly a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate or a modified glutamine synthetase as a target for a glutamine synthetase inhibitor such as phosphinotricin.

18. The plant of claim 13 wherein said marker DNA is: a gene encoding a protein or a polypeptide conferring a color to at least said specific tissue or specific cells, particularly the gene A1 or the GUS gene; or a gene encoding a protein or a polypeptide conferring a disease or pest resistance, particularly a gene encoding a *Bacillus thuringiensis* endotoxin that confers insect resistance or a gene encoding a bactericidal peptide that confers a bacterial resistance.

19. The plant of any one of claims 13 to 18, wherein said second promoter is: a constitutive promoter, particularly a PNOS promoter or a POCS promoter; a wound-inducible promoter, particularly a TR1' or TR2' promoter; a promoter which directs gene expression selectively in plant tissue having photosynthetic activity, particularly an SSU promoter; or a promoter which directs gene expression selectively in leaf cells, petal cells or seed cells, particularly seed coat cells.

20. The plant of claim 19 wherein said second promoter is a 35S promoter or a SSU promoter.

21. The plant of any one of claims 13 to 20, wherein said foreign DNA further comprises :

(f) a second DNA encoding a transit peptide capable of transporting said second protein or polypeptide into a chloroplast or mitochondria of at least said specific tissue or specific cells; said second DNA being in the same transcriptional unit as said marker DNA and said second promoter and between said marker DNA and said second promoter.

22. A plant which is a plant infectable by *Agrobacterium* and which contains, incorporated into the nuclear genome of all of its cells, the T-DNA of pTTM4 of Fig. 6, pTTM6 of Fig. 7A, pTTM6A- of Fig. 7B, pTTM8 of Fig. 8, pTVEP1 of Fig. 9A, pTVEP2 of Fig. 9B, pTVE62 of Fig. 10B or pTVE63 of Fig. 10A.

23. A culture of cells of the plant of any one of claims 1 to 22.

24. The promoter of the TA29 gene of Fig. 3A.

25. A DNA which contains a first chimeric DNA which comprises:

a) a male-sterility DNA encoding an RNA, protein or polypeptide, capable, when produced in stamen cells of a plant, of killing or disabling them to prevent the production of fertile male gametes; and
b) a first promoter which directs gene expression selectively in stamen cells of a plant, said DNA sequence being in the same transcriptional unit as, and under the control of, said first promoter,

provided that said first promoter is not a microspore and/or pollen specific promoter.

26. The DNA of claim 25, in which said first promoter directs expression of said male-sterility DNA in anther cells.

27. The DNA of claim 26, in which said first promoter directs expression of said male-sterility DNA in tapetum cells or anther epidermal cells of said plant.

28. The DNA of claim 27 wherein said first promoter is the promoter of the TA29 gene of Fig. 3A.

29. The DNA of claim 27 wherein said first promoter is the promoter of the endogenous tobacco TA26 gene encoding a mRNA from which a cDNA comprising the nucleotide sequence of Fig. 3C can be prepared, or the promoter of the endogenous tobacco TA13 gene encoding a mRNA from which a cDNA comprising the nucleotide sequence of Fig. 3B can be prepared, or a promoter of an endogenous gene of a plant coding for a tapetum-specific mRNA hybridizable to the TA29 gene of Fig. 3A, the said TA26 gene, or the said TA13 gene.

30. The DNA of any one of claims 25 to 29, wherein said male-sterility DNA encodes a ribonuclease, such as RNase T1.

31. The DNA of any one of claims 25 to 29 wherein said male-sterility DNA encodes a barnase.

32. The DNA of any one of claims 25 to 29, wherein said male-sterility DNA encodes: a DNase, especially an endonuclease, particularly EcoRI; a protease, especially a papain, particularly papain Zymogen or papain active protein; a glucanase; a lipase. particularly phospholipase A2; a lipid peroxidase; a cell wall inhibitor; a bacterial toxin; or a ribozyme, particularly the ribozyme against mRNA encoded by the TA29 gene of Fig. 3A, against mRNA of the TA26 gene from which a cDNA comprising the sequence of Fig. 3C can be prepared, or against mRNA from which a cDNA comprising the sequence of Fig. 3B can be prepared; or is an antisense DNA which encodes an RNA that is complementary to mRNA of said TA29 gene, said TA26 gene or said TA13 gene.

33. The DNA of any one of claims 25 to 29, wherein said male-sterility DNA encodes an enzyme which catalyzes the synthesis of a phytohormone, particularly the enzyme encoded by gene 4 of *Agrobacterium* T-DNA, or an enzyme encoded by gene 1 and/or gene 2 of *Agrobacterium* T-DNA.

34. The DNA of any one of claims 25 to 33 which also comprises :

(e) a first DNA encoding a transit peptide capable of transporting said first protein or polypeptide into a chloroplast or mitochondria of said stamen cells; said first DNA being in the same transcriptional unit as said male-sterility DNA and said first promoter and between said male-sterility DNA and said first promoter.

35. The DNA of claims 25 or 34, which also comprises a second chimeric DNA, comprising :

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or in at least specific cells of a plant, renders said plant easily separable from other plants which do not contain said second RNA, protein or polypeptide in said specific tissue or specific cells; and
(d) a second promoter capable of directing expression of said marker DNA at least in said specific tissue or specific cells; said marker DNA being in the same transcriptional unit as, and under the control of, said second promoter.

36. The DNA of claims 35 wherein said marker DNA encodes a protein inhibiting or neutralizing the action of a herbicide.

37. The DNA of claim 36 in which said marker DNA is an herbicide resistance gene, particularly a gene conferring resistance to a glutamine synthetase inhibitor, such as phosphinothricin.

38. The DNA of claim 37 in which said marker DNA is a sfr or sfrv gene.

39. The DNA of claim 36, wherein said marker DNA is a gene encoding a modified target enzyme for an herbicide having lower affinity for the herbicide, particularly a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate or a modified glutamine synthetase as a target for a glutamine synthetase inhibitor such as phosphinotricin.

40. The DNA of claim 35, wherein said marker DNA is a gene encoding a protein or a polypeptide conferring a color to at least said specific tissue or specific cells, particularly the gene A1 or the GUS gene; or a gene encoding a protein or a polypeptide conferring a disease or pest resistance, particularly a gene encoding a *Bacillus thuringiensis* endotoxin that confers insect resistance or a gene encoding a bactericidal peptide that confers a bacterial resistance.

41. The DNA of claim 35 wherein said second promoter is: a constitutive promoter, particularly a PNOS promoter or a POCS promoter; a wound-inducible promoter, particularly a TR1' or TR2' promoter; a promoter which directs gene expression selectively in plant tissue having photosynthetic activity, or a promoter which directs gene expression selectively in leaf cells, petal cells or seed cells, particularly seed coat cells.

42. The DNA of claim 41 wherein said second promoter is a 35S promoter or an SSU promoter.

43. The DNA of any one of claims 35 to 42 which also comprises :

(f) a second DNA encoding a transit peptide capable of transporting said second protein or polypeptide into a chloroplast or mitochondria of at least said specific tissue or specific cells; said second DNA being in the same transcriptional unit as said marker DNA and said second promoter and between said marker DNA and said second promoter.

44. A DNA which is the T-DNA of pTTM4 of Fig. 6, pTTM6 of Fig. 7A, pTTM6A- of Fig. 7B, pTTM8 of Fig. 8, pTVEP1 of Fig. 9A, pTVEP2 of Fig. 9B, pTVE62 of Fig. 10B or pTVE63 of Fig. 10A.

45. The DNA of any one of claims 25 to 44 which is nuclear DNA of a cell of a plant or of a seed.

46. A vector which comprises the DNA of any one of claims 25 to 44.

47. A cell of a plant which contains the DNA of any one of claims 25 to 45.

48. A cell of a plant which contains, stably integrated into its nuclear DNA, the DNA of any one of claims 25 to 44.

49. A cell of claim 47 or claim 48 which can be regenerated into a plant which is male-sterile.

50. The cell of any one of claims 47 to 49 wherein said DNA comprises a male-sterility DNA that encodes a ribonuclease.

51. The cell of any one of claims 47 to 49 wherein said DNA comprises a male-sterility DNA that encodes a barnase.

52. The cell of any one of claims 47 to 51 wherein said DNA comprises a first promoter which directs expression in anther cells.

53. The cell of any one of claims 47 to 52 wherein said DNA comprises a first promoter which directs expression in tapetum cells.

54. The cell of any one of claims 47 to 53 wherein said DNA comprises a first promoter which is the promoter of the TA29 gene of Fig. 3A.

55. The cell of any one of claims 47 to 54 in which said DNA comprises a herbicide resistance gene under control of a second promoter which is a constitutive promoter or of a promoter that directs gene expression selectively in plant tissue having photosynthetic activity.

56. The cell of claim 55 in which said herbicide resistance gene confers resistance to a glutamine synthetase inhibitor such as phosphinothricin.

57. The cell of claim 56 in which said herbicide resistance gene is the sfr or sfrv gene.

58. The cell of any one of claims 55 to 57 in which said second promoter is a 35S promoter or an SSU promoter.

59. A plant which contains the DNA of any one of claims 25 to 45.

60. A plant which contains the DNA of any one of claims 25 to 45 in all of its cells.

61. The plant of claim 59 or 60 which is male-sterile.

62. The plant of any one of claims 59 to 61 which is a hybrid plant.

63. A plant seed which contains the DNA of any one of claims 25 to 45.

64. A process for producing a male-sterile plant and reproduction materials, e.g. seeds of said male-sterile plant, which comprises introducing the DNA of any one of claims 25 to 44 into the nuclear genome of a plant cell to thereby

obtain a transformed plant cell, regenerating said male-sterile plant from said transformed plant cell, and, optionally obtaining from said male-sterile plant said reproduction material or progeny which contains said foreign DNA.

65. A process for producing a seed of a plant which is a seed-forming and male-sterile plant, which comprises:

- cross-pollinating i) plants of any one of claims 14 to 22, or plants containing, stably integrated into the nuclear DNA of all of their cells, the DNA of any one of claims 36 to 44, which are seed-forming plants and are male-sterile, in which said marker DNA is a gene conferring resistance to a herbicide or a gene encoding a modified target enzyme for said herbicide, and ii) male-fertile plants without said marker DNA and second promoter,
- applying said herbicide to the plants for eliminating male-fertile plants,
- obtaining seeds of said pollinated male-sterile plants.

66. The process of claim 65 which comprises applying said herbicide prior to said cross-pollination.

67. The process of claim 65 which comprises applying said herbicide after said cross-pollination.

68. The process of any one of claims 65 to 67 in which said marker DNA is a gene capable of conferring resistance to a glutamine synthetase inhibitor such as phosphinotricin, particularly the sfr or sfrv gene, and which comprises applying said synthetase inhibitor to the plants.

69. The process of any one of claims 65 to 68 wherein the male-sterile plants contain in addition to said marker DNA a second marker DNA, particularly another herbicide resistance gene or a gene encoding a modified target enzyme for another herbicide, stably integrated into the nuclear genome of their cells in the same genetic locus as said male-sterility DNA; and wherein said male-fertile plants used for pollination contain only the second marker DNA stably integrated in the nuclear genome of their cells.

70. The process of any one of claims 65 to 69 wherein said male-sterile plants and said male-fertile plants are capable of giving rise to hybrid plants, and which comprises recovering hybrid seeds of said male-sterile plants.

71. A process to maintain an inbred line of plants containing, stably integrated into the nuclear DNA of all of their cells, the DNA of any one of claims 35 to 44, which plants are seed-forming male-sterile plants that are heterozygous for said DNA, and in which said marker DNA is gene conferring resistance to a herbicide or a gene encoding a modified target enzyme for said herbicide, said process comprising:

cross-pollinating i) male-sterile plants of said inbred line, and ii) male-fertile plants of that inbred line without said marker DNA and said second promoter,
and, after said cross-pollination,
obtaining seeds from said male-sterile plants,
growing said seeds into plants and
applying said herbicide to the plants for eliminating male-fertile plants.

72. The process of claim 71 in which said marker DNA is a gene capable of conferring resistance to a glutamine synthetase inhibitor, such as phosphinotricin, particularly a sfr or sfrv gene, and which comprises applying said glutamine synthetase inhibitor the plants.

73. A pair of parent plants for producing seeds comprising : a) a male-sterile parent plant containing the DNA of any one of claims 25 to 45, and b) a male-fertile parent plant.

74. The pair of parent plants of claim 73 wherein said male-sterile parent plant and male-fertile parent plant belong to different lines.

75. The pair of parent plants of claim 73 wherein said male-sterile parent plant and male-fertile parent plant are derived from the same inbred line.

76. The pair of parent plants of any one of claims 74 to 76 wherein said DNA comprises a male-sterility DNA that encodes a ribonuclease.

77. The pair of parent plants of any one of claims 74 to 76 wherein said DNA comprises a male-sterility DNA that

encodes barnase.

78. The pair of parent plants of any one of claims 74 to 78 wherein said DNA comprises a first promoter which directs expression in anther cells.

79. The pair of parent plants of any one of claims 74 to 79 wherein said DNA comprises a first promoter which directs expression in tapetum cells.

80. The pair of parent plants of any one of claims 74 to 79 wherein said DNA comprises a first promoter which is the promoter of the TA29 gene of Fig. 3A.

81. The pair of parent plants of any one of claims 74 to 81 in which said DNA comprises a herbicide resistance gene under control of a second promoter which is a constitutive promoter or of a promoter that directs gene expression selectively in plant tissue having photosynthetic activity.

82. The pair of parent plants of claim 82 in which said herbicide resistance gene confers resistance to a glutamine synthetase inhibitor such as phosphinothricin.

83. The pair of parent plants of claim 83 in which said herbicide resistance gene is the sfr or sfrv gene.

84. The pair of parent plants of any one of claims 82 to 84 in which said second promoter is a 35S promoter or an SSU promoter.

Patentansprüche

1. Pollensterile Pflanze, die eine fremde DNA enthält, die in dem Kerngenom sämtlicher ihrer Zellen enthalten ist, wobei die fremde DNA umfaßt:

   (a) eine Pollensterilitäts-DNA, die eine erste RNA oder ein erstes Protein oder Polypeptid kodiert, die bzw. das, wenn sie bzw. es in Staubblattzellen der Pflanze hergestellt wird, diese töten oder schädigen kann, um die Produktion fertiler männlicher Gameten zu verhindern,
   (b) einen ersten Promotor, der eine Genexpression selektiv in Staubblattzellen der Pflanze bewirkt, wobei die Pollensterilitäts-DNA sich in derselben Transkriptionseinheit befindet wie der erste Promotor und unter dessen Kontrolle steht,

   vorausgesetzt, daß, wenn der erste Promotor ein Promotor ist, der eine Expression der Pollensterilitäts-DNA selektiv in Mikrosporen- und/oder Pollenzellen bewirkt, das Kerngenom der Zellen der Pflanze homozygot ist.

2. Pflanze nach Anspruch 1,
   in der der erste Promotor eine Expression der Pollensterilitäts-DNA in Antherenzellen bewirkt.

3. Pflanze nach Anspruch 2,
   in der der erste Promotor eine Expression der Pollensterilitäts-DNA in Tapetumzellen oder Antheren-Epidermiszellen der Pflanze bewirkt.

4. Pflanze nach einem der Ansprüche 1 bis 3,
   in der der erste Promotor der Promotor des TA29-Gens aus Fig. 3A ist.

5. Pflanze nach einem der Ansprüche 1 bis 3,
   in der der erste Promotor der Promotor des endogenen TA26-Gens aus Tabak, das eine mRNA kodiert, aus der eine cDNA, die die Nukleotidsequenz der Fig. 3C umfaßt, hergestellt werden kann, der Promotor des endogenen TA13-Gens aus Tabak, das eine mRNA kodiert, aus der eine cDNA, die die Nukleotidsequenz der Fig. 3B umfaßt, hergestellt werden kann, oder ein Promotor eines endogenen Gens einer Pflanze ist, das eine tapetumspezifische mRNA kodiert, die mit dem TA29-Gen, dem TA26-Gen oder dem TA13-Gen hybridisieren kann.

6. Pflanze nach Anspruch 1,
   die eine homozygote Pflanze ist und in der der erste Promotor eine Expression der Pollensterilitäts-DNA selektiv

in Pollenzellen der homozygoten Pflanze bewirkt.

7. Pflanze nach einem der Ansprüche 1 bis 6,
in der die Pollensterilitäts-DNA eine RNase, insbesondere RNase T1, kodiert.

8. Pflanze nach einem der Ansprüche 1 bis 6,
in der die Pollensterilitäts-DNA Barnase kodiert.

9. Pflanze nach einem der Ansprüche 1 bis 6,
in der die Pollensterilitäts-DNA kodiert: eine DNase, insbesondere eine Endonuklease, besonders EcoRI, eine Protease, insbesondere ein Papain, besonders Papain-Zymogen oder aktives Papainprotein, eine Glucanase, eine Lipase, insbesondere Phospholipase A2, eine Lipidperoxidase, einen Zellwandinhibitor, ein bakterielles Toxin, insbesondere das A-Fragment des Diphtheria-Toxins, oder ein Ribozym, insbesondere das Ribozym gegen von dem TA29-Gen der Fig. 3A kodierte mRNA, gegen mRNA des TA26-Gens, aus dem eine die Sequenz der Fig. 3C umfassende cDNA hergestellt werden kann, oder gegen mRNA, aus der eine die Sequenz der Fig. 3B umfassende cDNA hergestellt werden kann, oder eine antisense-DNA ist, die eine RNA kodiert, die zu mRNA des TA29-Gens, des TA26-Gens oder des TA13-Gens komplementär ist.

10. Pflanze nach einem der Ansprüche 1 bis 6,
in der die Pollensterilitäts-DNA ein Enzym, das die Synthese eines Phytohormons katalysiert, insbesondere das von Gen 4 von *Agrobacterium*-T-DNA kodierte Enzym oder ein Enzym, das von Gen 1 und/oder Gen 2 von *Agrobacterium*-T-DNA kodiert wird, kodiert.

11. Pflanze nach einem der Ansprüche 1 bis 10,
in der die fremde DNA ferner umfaßt:
(e) eine erste DNA, die ein Transitpeptid kodiert, das das erste Protein oder Polypeptid in einen Chloroplasten oder in Mitochondrien der Staubblattzellen transportieren kann, wobei die erste DNA sich in derselben Transkriptionseinheit wie die Pollensterilitats-DNA und der erste Promotor und zwischen der Pollensterilitäts-DNA und dem ersten Promotor befindet.

12. Pflanze nach einem der Ansprüche 1 bis 11,
die aus der Gruppe von Mais, Kartoffel, Tomate, Ölsamenraps, Alfalfa, Sonnenblume, Baumwolle, Sellerie, Zwiebel, Klee, Sojabohne, Tabak, Brassica-Gemüsen oder Zuckerrübe ausgewählt ist.

13. Pflanze nach einem der Ansprüche 1 bis 12,
in der die fremde DNA gleichfalls umfaßt:

(c) eine Marker-DNA, die eine zweite RNA oder ein zweites Protein oder Polypeptid kodiert, die bzw. das, wenn sie bzw. es zumindest in einem spezifischen Gewebe oder in mindestens spezifischen Zellen der Pflanze vorkommt, die Pflanze leicht von anderen Pflanzen abtrennbar macht, die die zweite RNA oder das zweite Protein oder Polypeptid in dem spezifischen Gewebe oder in den spezifischen Zellen nicht enthalten, und
(d) einen zweiten Promotor, der eine Expression der Marker-DNA zumindest in dem spezifischen Gewebe oder in den spezifischen Zellen bewirken kann, wobei die Marker-DNA sich in derselben Transkriptionseinheit wie der zweite Promotor befindet und unter dessen Kontrolle steht.

14. Pflanze nach Anspruch 13,
in der die Marker-DNA ein Protein kodiert, das die Wirkung eines Herbizids hemmt oder neutralisiert.

15. Pflanze nach Anspruch 14,
in der die Marker-DNA ein Herbizidresistenzgen, insbesondere ein Gen, das eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, vermittelt, ist.

16. Pflanze nach Anspruch 15,
in der die Marker-DNA ein sfr- oder sfrv-Gen ist.

17. Pflanze nach Anspruch 14,
in der die Marker-DNA ein modifiziertes Zielenzym für ein Herbizid kodiert, das eine geringere Alfinität zu dem Herbizid hat, insbesondere eine modifizierte 5-Enolpyruvylshikimat-3-phosphat-Synthase als Ziel für Glyphosat

oder eine modifizierte Glutamin-Synthetase als Ziel für einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin.

18. Pflanze nach Anspruch 13,
in der die Marker-DNA ist: ein Gen, das ein Protein oder ein Polypeptid kodiert, das zumindest dem spezifischen Gewebe oder den spezifischen Zellen eine Farbe vermittelt, insbesondere das Gen A1 oder das GUS-Gen,
oder ein Gen, das ein Protein oder Polypeptid kodiert, das eine Krankheits- oder Schädlingsresistenz vermittelt, insbesondere ein Gen, das ein *Bacillus thuringiensis*-Endotoxin kodiert, das eine Insektenresistenz vermittelt, oder ein Gen, das ein bakterielles Peptid kodiert, das eine Bakterienresistenz vermittelt.

19. Pflanze nach einem der Ansprüche 13 bis 18,
in der der zweite Promotor ein konstitutiver Promotor, insbesondere ein PNOS-Promotor oder ein POCS-Promotor, ein wundinduzierbarer Promotor, insbesondere ein TR1'- oder TR2'- Promotor, ein Promotor, der eine Genexpression selektiv in Pflanzengewebe mit photosynthetischer Aktivität bewirkt, insbesondere ein SSU-Promotor, oder ein Promotor, der eine Genexpression selektiv in Blattzellen, Petalzellen oder Samenzellen, insbesondere Samenschalenzellen, bewirkt, ist.

20. Pflanze nach Anspruch 19,
in der der zweite Promotor ein 35S-Promotor oder ein SSU-Promotor ist.

21. Pflanze nach einem der Ansprüche 13 bis 20,
in der die fremde DNA ferner umfaßt:
(f) eine zweite DNA, die ein Transitpeptid kodiert, das das zweite Protein oder Polypeptid in einen Chloroplasten oder in Mitochondrien von zumindest dem spezifischen Gewebe oder den spezifischen Zellen transportieren kann, wobei sich die zweite DNA in derselben Transkriptionseinheit wie die Marker-DNA und der zweite Promotor und zwischen der Marker-DNA und dem zweiten Promotor befindet.

22. Pflanze,
die eine durch *Agrobacterium* infizierbare Pflanze ist und die enthält, eingebaut in das Kerngenom sämtlicher ihrer Zeilen, die T-DNA von pTTM4 von Fig. 6, pTTM6 von Fig. 7A. pTTM6A' von Fig. 7B, pTTM8 von Fig. 8, pTVEP1 von Fig. 9A, pTVEP2 von Fig. 9B, pTVE62 von Fig. 10B oder pTVE63 von Fig. 10A.

23. Kultur von Zellen der Pflanze nach einem der Ansprüche 1 bis 22.

24. Promotor des TA29-Gens von Fig. 3A.

25. DNA,
die eine erste chimäre DNA enthält, die umfaßt:

a) eine Pollensterilitäts-DNA, die eine RNA oder ein Protein oder Polypeptid kodiert, die bzw. das, wenn sie bzw. es in Staubblattzellen einer Pflanze hergestellt wird, diese töten oder schädigen kann, um die Produktion fertiler männlicher Gameten zu verhindern, und
b) einen ersten Promotor, der eine Genexpression selektiv in Staubblattzellen einer Pflanze bewirkt, wobei die DNA-Sequenz sich in derselben Transkriptionseinheit wie der erste Promotor befindet und unter dessen Kontrolle steht,

vorausgesetzt, daß der erste Promotor kein Mikrosporen- und/oder Pollen-spezifischer Promotor ist.

26. DNA nach Anspruch 25,
in der der erste Promotor eine Expression der Pollensterilitäts-DNA in Antherenzellen bewirkt.

27. DNA nach Anspruch 26,
in der der erste Promotor eine Expression der Pollensterilitäts-DNA in Tapetumzellen oder Antheren-Epidermiszellen der Pflanze bewirkt.

28. DNA nach Anspruch 27,
in der der erste Promotor der Promotor des TA29-Gens von Fig. 3A ist.

29. DNA nach Anspruch 27,

in der der erste Promotor der Promotor des endogenen TA26-Gens aus Tabak, das eine mRNA kodiert, aus der eine die Nukleotidsequenz der Fig. 3C umfassende cDNA hergestellt werden kann, oder der Promotor des endogenen TA13-Gens aus Tabak, das eine mRNA kodiert, aus der eine die Nukleotidsequenz der Fig. 3B umfassende cDNA hergestellt werden kann, oder ein Promotor eines endogenen Gens einer Pflanze ist, das eine tapetumspezifische mRNA kodiert, die mit dem TA29-Gen der Fig. 3A, dem TA26-Gen oder dem TA13-Gen hybridisieren kann.

30. DNA nach einem der Ansprüche 25 bis 29,
in der die Pollensterilitäts-DNA eine Ribonuklease, wie RNase T1, kodiert.

31. DNA nach einem der Ansprüche 25 bis 29,
in der die Pollensterilitäts-DNA eine Barnase kodiert.

32. DNA nach einem der Ansprüche 25 bis 29,
in der die Pollensterilitäts-DNA kodiert: eine DNase, insbesondere eine Endonuklease, besonders EcoRI, eine Protease, insbesondere ein Papain, besonders Papain-Zymogen oder aktives Papainprotein, eine Glucanase, eine Lipase, insbesondere Phospholipase A2, eine Lipidperoxidase, einen Zellwandinhibitor, ein bakterielles Toxin oder ein Ribozym, insbesondere das Ribozym gegen von dem TA29-Gen der Fig. 3A kodierte mRNA, gegen mRNA des TA26-Gens, aus dem eine die Sequenz der Fig. 3C umfassende cDNA hergestellt werden kann, oder gegen mRNA, aus der eine die Sequenz der Fig. 3B umfassende cDNA hergestellt werden kann, oder eine antisense-DNA ist, die eine RNA kodiert, die zu mRNA des TA29-Gens, des TA26-Gens oder des TA13-Gens komplementär ist.

33. DNA nach einem der Ansprüche 25 bis 29,
in der die Pollensterilitäts-DNA ein Enzym das die Synthese eines Phytohormons katalysiert, insbesondere das von Gen 4 von *Agrobacterium*-T-DNA kodierte Enzym oder ein Enzym, das von Gen 1 und/oder Gen 2 von *Agrobacterium*-T-DNA kodiert wird, kodiert.

34. DNA nach einem der Ansprüche 25 bis 33,
die gleichfalls umfaßt:

(e) eine erste DNA, die ein Transitpeptid kodiert, das das erste Protein oder Polypeptid in einen Chloroplasten oder in Mitochondrien der Staubblattzellen transportieren kann, wobei die erste DNA sich in derselben Transkriptionseinheit wie die Pollensterilitäts-DNA und der erste Promotor und zwischen der Pollensterilitäts-DNA und dem ersten Promotor befindet.

35. DNA nach den Ansprüchen 25 oder 34,
die gleichfalls eine zweite chimäre DNA enthält, die umfaßt:

(c) eine Marker-DNA, die eine zweite RNA oder ein zweites Protein oder Polypeptid kodiert, die bzw. das, wenn sie bzw. es zumindest in einem spezifischen Gewebe oder in zumindest spezifischen Zellen einer Pflanze vorkommt, die Pflanze leicht von anderen Pflanzen abtrennbar macht, die die zweite RNA oder das zweite Protein oder Polypeptid in dem spezifischen Gewebe oder in den spezifischen Zellen nicht enthalten, und
(d) einen zweiten Promotor, der eine Expression der Marker-DNA zumindest in dem spezifischen Gewebe oder in den spezifischen Zellen bewirken kann, wobei die Marker-DNA sich in derselben Transkriptionseinheit wie der zweite Promotor befindet und unter dessen Kontrolle steht.

36. DNA nach Anspruch 35,
in der die Marker-DNA ein Protein kodiert, das die Wirkung eines Herbizids hemmt oder neutralisiert.

37. DNA nach Anspruch 36,
in der die Marker-DNA ein Herbizidresistenzgen, insbesondere ein Gen, das eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, vermittelt, ist.

38. DNA nach Anspruch 37,
in der die Marker-DNA ein sfr- oder sfrv-Gen ist.

39. DNA nach Anspruch 36,
in der die Marker-DNA ein Gen ist, das ein modifiziertes Zielenzym für ein Herbizid, das eine geringere Affinität zu dem Herbizid hat, insbesondere eine modifizierte 5-Enolpyruvylshikimat-3-phosphat-Synthase als Ziel für Gly-

phosat oder eine modifizierte Glutamin-Synthetase als Ziel für einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, kodiert.

40. DNA nach Anspruch 35,
in der die Marker-DNA ein Gen, das ein Protein oder ein Polypeptid kodiert, das zumindest dem spezifischen Gewebe oder den spezifischen Zellen eine Farbe vermittelt, insbesondere das Gen AI oder das GUS-Gen, oder ein Gen ist, das ein Protein oder Polypeptid kodiert, das eine Krankheits- oder Schädlingsresistenz vermittelt, insbesondere ein Gen, das ein *Bacillus thuringiensis*-Endotoxin kodiert, das eine Insektenresistenz vermittelt, oder ein Gen, das ein bakterielles Peptid kodiert, das eine Bakterienresistenz vermittelt.

41. DNA nach Anspruch 35,
in der der zweite Promotor ein konstitutiver Promotor, insbesondere ein PNOS-Promotor oder ein POCS-Promotor, ein wundinduzierbarer Promotor, insbesondere ein TR1'- oder TR2'- Promotor, ein Promotor, der eine Genexpression selektiv in Pflanzengewebe mit photosynthetischer Aktivität bewirkt, oder ein Promotor, der eine Genexpression selektiv in Blattzellen, Petalzellen oder Samenzellen, insbesondere Samenschalenzellen, bewirkt, ist.

42. DNA nach Anspruch 41,
in der der zweite Promotor ein 35S-Promotor oder ein SSU-Promotor ist.

43. DNA nach einem der Ansprüche 25 bis 42,
die gleichfalls umfaßt:
(f) eine zweite DNA, die ein Transitpeptid kodiert, das das zweite Protein oder Polypeptid in einen Chloroplasten oder in Mitochondrien von zumindest dem spezifischen Gewebe oder den spezifischen Zellen transportieren kann, wobei sich die zweite DNA in derselben Transkriptionseinheit wie die Marker-DNA und der zweite Promotor und zwischen der Marker-DNA und dem zweiten Promotor befindet.

44. DNA,
die die T-DNA von pTTM4 von Fig. 6, pTTM6 von Fig. 7A, pTTM6A⁻ von Fig. 7B, pTTM8 von Fig. 8, pTVEP1 von Fig. 9A, pTVEP2 von Fig. 9B, pTVE62 von Fig. 10B oder pTVE63 von Fig. 10A ist.

45. DNA nach einem der Ansprüche 25 bis 44,
die eine Zellkern-DNA einer Zelle einer Pflanze oder eines Samens ist.

46. Vektor,
der die DNA nach einem der Ansprüche 25 bis 44 umfaßt.

47. Pflanzenzelle, die die DNA nach einem der Ansprüche 25 bis 45 umfaßt.

48. Pflanzenzelle, die stabil in ihre Zellkern-DNA integriert die DNA nach einem der Ansprüche 25 bis 44 enthält.

49. Zelle nach Anspruch 47 oder Anspruch 48,
die zu einer Pflanze, die pollensteril ist, regeneriert werden kann.

50. Zelle nach einem der Ansprüche 47 bis 49,
in der die DNA eine Pollensterilitäts-DNA, die eine Ribonuklease kodiert, umfaßt.

51. Zelle nach einem der Ansprüche 47 bis 49,
in der die DNA eine Pollensterilitäts-DNA, die eine Barnase kodiert, umfaßt.

52. Zelle nach einem der Ansprüche 47 bis 51,
in der die DNA einen ersten Promotor, der eine Expression in Antherenzellen bewirkt, umfaßt.

53. Zelle nach einem der Ansprüche 47 bis 52,
in der die DNA einen ersten Promotor, der eine Expression in Tapetumzellen bewirkt, umfaßt.

54. Zelle nach einem der Ansprüche 47 bis 53,
in der die DNA einen ersten Promotor, der der Promotor des TA29-Gens der Fig. 3A ist, umfaßt.

55. Zelle nach einem der Ansprüche 47 bis 54,
in der die DNA ein Herbizidresistenzgen unter der Kontrolle eines zweiten Promotors, der ein konstitutiver Promotor ist, oder unter der Kontrolle eines Promotors, der eine Genexpression selektiv in Pflanzengewebe mit photosynthetischer Aktivität bewirkt, umfaßt.

56. Zelle nach Anspruch 55,
in der das Herbizidresistenzgen eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie phosphinothricin, vermittelt.

57. Zelle nach Anspruch 56,
in der das Herbizidresistenzgen das sfr- oder sfrv-Gen ist.

58. Zelle nach einem der Ansprüche 55 bis 57,
in der der zweite Promotor ein 35S-Promotor oder ein SSU-Promotor ist.

59. Pflanze,
die die DNA nach einem der Ansprüche 25 bis 45 enthält.

60. Pflanze,
die die DNA nach einem der Ansprüche 25 bis 45 in sämtlichen ihrer Zellen enthält.

61. Pflanze nach Anspruch 59 oder 60,
die pollensteril ist.

62. Pflanze nach einem der Ansprüche 59 bis 61,
die eine hybride Pflanze ist.

63. Pflanzensamen,
der die DNA nach einem der Ansprüche 25 bis 45 enthält.

64. Verfahren zum Herstellen einer pollensterilen Pflanze und von Reproduktionsmaterialien, z.B. Samen, der pollensterilen Pflanze,
das umfaßt, die DNA nach einem der Ansprüche 25 bis 44 in das Kerngenom einer Pflanzenzelle einzuführen, um dadurch eine transformierte Pflanzenzelle zu erhalten, die pollensterile Pflanze aus der transformierten Pflanzenzelle zu regenerieren und gegebenenfalls von der pollensterilen Pflanze das Reproduktionsmaterial oder die Nachkommenschaft, das bzw. die die fremde DNA enthält, zu erhalten.

65. Verfahren zum Herstellen eines Samens einer Pflanze die eine samenbildende und pollenstenle Pflanze ist, das umfaßt:

- Kreuzbestäuben i) von Pflanzen nach einem der Ansprüche 14 bis 22 oder von Pflanzen, die stabil in die Zellkern-DNA sämtlicher ihrer Zellen integriert die DNA nach einem der Ansprüche 36 bis 44 enthalten, die samenbildende Pflanzen und pollensteril sind, in denen die Marker-DNA ein Gen, das eine Resistenz gegen ein Herbizid vermittelt, oder ein Gen, das ein modifiziertes Zielenzym für das Herbizid kodiert, ist, und ii) von pollenfertilen Pflanzen ohne die Marker-DNA und den zweiten Promotor,
- Aufbringen des Herbizids auf die Pflanzen, um pollenfertile Pflanzen zu entfernen,
- Gewinnen von Samen von den bestäubten pollensterilen Pflanzen.

66. Verfahren nach Anspruch 65,
das ein Aufbringen des Herbizids vor der Kreuzbestäubung umfaßt.

67. Verfahren nach Anspruch 65,
das ein Aufbringen des Herbizids nach der Kreuzbestäubung umfaßt.

68. Verfahren nach einem der Ansprüche 65 bis 67,
in dem die Marker-DNA ein Gen, das eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, vermitteln kann, insbesondere das sfr- oder sfrv-Gen ist und das Verfahren ein Aufbringen des Synthetase-Inhibitors auf die Pflanzen umfaßt.

69. Verfahren nach einem der Ansprüche 65 bis 68,
in dem die pollensterilen Pflanzen zusätzlich zu der Marker-DNA eine zweite Marker-DNA enthalten, insbesondere ein anderes Herbizidresistenzgen oder ein Gen, das ein modifiziertes Zielenzym für ein anderes Herbizid kodiert, die stabil in das Kerngenom ihrer Zellen in demselben genetischen Locus wie die Pollensterilitäts-DNA integriert ist, und wobei die für eine Bestäubung verwendeten pollenfertilen Pflanzen nur die zweite Marker-DNA stabil in das Kerngenom ihrer Zellen integriert enthalten.

70. Verfahren nach einem der Ansprüche 65 bis 69,
in dem die pollensterilen Pflanzen und die pollenfertilen Pflanzen in der Lage sind, hybride Pflanzen zu erzeugen, und das Verfahren umfaßt, hybride Samen von den pollensterilen Pflanzen zu gewinnen.

71. Verfahren zum Aufrechterhalten einer Inzuchtlinie von Pflanzen, die stabil in die Zellkern-DNA sämtlicher ihrer Zellen integriert die DNA nach einem der Ansprüche 35 bis 44 enthalten, wobei die Pflanzen samenbildende pollensterile Pflanzen sind, die bezüglich dieser DNA heterozygot sind, und in dem die Marker-DNA ein Gen, das eine Resistenz gegen ein Herbizid vermittelt, oder ein Gen, das ein modifiziertes Zielenzym für das Herbizid kodiert, ist, wobei das Verfahren umfaßt:

- Kreuzbestäuben von i) pollensterilen Pflanzen der Inzuchtlinie und ii) pollenfertilen Pflanzen der Inzuchtlinie ohne die Marker-DNA und den zweiten Promotor und nach dem Kreuzbestäuben
- Gewinnen von Samen von den pollensterilen Pflanzen,
- Züchten der Samen zu Pflanzen und
- Aufbringen des Herbizids auf die Pflanzen, um pollenfertile Pflanzen zu entfernen.

72. Verfahren nach Anspruch 71,
in dem die Marker-DNA ein Gen, das eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, vermitteln kann, insbesondere ein sfr- oder ein sfrv-Gen, ist, und das ein Aufbringen des Glutamin-Synthetase-Inhibitors auf die Pflanzen umfaßt.

73. Paar von Elternpflanzen zum Produzieren von Samen,
umfassend: a) eine pollensterile Elternpflanze, die die DNA nach einem der Ansprüche 25 bis 45 enthält, und b) eine pollenfertile Elternpflanze.

74. Paar von Elternpflanzen nach Anspruch 73,
in dem die pollensterile Elternpflanze und die pollenfertile Elternpflanze zu unterschiedlichen Linien gehören.

75. Paar von Elternpflanzen nach Anspruch 73,
in dem die pollensterile Elternpflanze und die pollenfertile Elternpflanze von derselben Inzuchtlinie abgeleitet sind.

76. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 75,
in dem die DNA eine eine Ribonuklease kodierende Pollensterilitäts-DNA umfaßt.

77. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 75,
in dem die DNA eine Barnase kodierende Pollensterilitäts-DNA umfaßt.

78. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 77,
in dem die DNA einen ersten Promotor umfaßt, der eine Expression in Antherenzellen bewirkt.

79. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 78,
in dem die DNA einen ersten Promotor umfaßt, der eine Expression in Tapetumzellen bewirkt.

80. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 78,
in dem die DNA einen ersten Promotor umfaßt, der der Promotor des TA29-Gens von Fig. 3A ist.

81. Paar von Elternpflanzen nach einem der Ansprüche 73 bis 80,
in dem die DNA ein Herbizidresistenzgen unter der Kontrolle eines zweiten Promotors, der ein konstitutiver Promotor ist, oder unter der Kontrolle eines Promotors, der eine Genexpression selektiv in Pflanzengewebe mit photosynthetischer Aktivität bewirkt, umfaßt.

82. Paar von Elternpflanzen nach Anspruch 81, in dem das Herbizidresistenzgen eine Resistenz gegen einen Glutamin-Synthetase-Inhibitor, wie Phosphinothricin, vermittelt.

83. Paar von Elternpflanzen nach Anspruch 82, in dem das Herbizidresistenzgen das sfr- oder sfrv-Gen ist.

84. Paar von Elternpflanzen nach einem der Ansprüche 81 bis 83, in dem der zweite Promotor ein 35S-Promotor oder ein SSU-Promotor ist.

Revendications

1. Plante à stérilité mâle contenant un ADN étranger incorporé dans le génome nucléaire de la totalité de ses cellules, ledit ADN étranger comprenant:

   (a) un ADN de stérilité mâle codant pour un premier ARN, protéine ou polypeptide capable, lorsqu'il est produit dans les cellules des étamines de ladite plante, de les tuer ou de les rendre incompétentes pour empêcher la production de gamètes mâles fertiles;
   (b) un premier promoteur qui dirige l'expression génique sélectivement dans les cellules des étamines de ladite plante, ledit ADN de stérilité mâle étant dans la même unité de transcription que ledit premier promoteur, et étant sous son contrôle,

   à la condition que, si ledit premier promoteur est un promoteur qui dirige l'expression dudit ADN de stérilité mâle sélectivement dans les cellules des microspores et/ou du pollen, le génome nucléaire des cellules de ladite plante soit homozygote.

2. Plante selon la revendication 1, dans laquelle ledit premier promoteur dirige l'expression dudit ADN de stérilité mâle dans les cellules d'anthère.

3. Plante selon la revendication.2, dans laquelle ledit premier promoteur dirige l'expression dudit ADN de stérilité mâle dans les cellules de tapétum ou dans les cellules épidermiques de l'anthère de ladite plante.

4. Plante selon l'une quelconque des revendications 1 à 3, dans laquelle ledit premier promoteur est le promoteur du gène TA29 de la figure 3A.

5. Plante selon l'une quelconque des revendications 1 à 3, dans laquelle ledit premier promoteur est le promoteur du gène endogène du tabac TA26 codant pour un ARNm à partir duquel on peut préparer un ADNc comprenant la séquence nucléotidique de la figure 3C, le promoteur du gène endogène du tabac TA13 codant pour un ARNm à partir duquel on peut préparer un ADNc comprenant la séquence nucléotidique de la figure 3B, ou un promoteur d'un gène endogène d'une plante codant pour un ARNm spécifique au tapétum susceptible d'être hybridé audit gène TA29, audit gène TA26 ou audit gène TA13.

6. Plante selon la revendication 1, qui est une plante homozygote et dans laquelle ledit premier promoteur dirige l'expression dudit ADN de stérilité mâle sélectivement dans les cellules du pollen de ladite plante homozygote.

7. Plante selon l'une quelconque des revendications 1 à 6, dans laquelle ledit ADN de stérilité mâle code pour une ARNase, en particulier l'ARNase T1.

8. Plante selon l'une quelconque des revendications 1 à 6, dans laquelle ledit ADN de stérilité mâle code pour le barnase.

9. Plante selon l'une quelconque des revendications 1 à 6, dans laquelle ledit ADN de stérilité mâle code pour: une ADNase, en particulier une endonucléase, tout spécialement EcoRI; une protéase, en particulier une papaïne, tout spécialement le zymogène de papaïne ou une protéine à activité de papaïne; une glucanase, une lipase, en particulier la phospholipase A2; une peroxydase lipidique; un inhibiteur de paroi cellulaire; une toxine bactérienne, en particulier le fragment A de la toxine diphtérique; ou un ribozyme, en particulier le ribozyme contre l'ARNm codé par le gène TA29 de la figure 3A, contre l'ARNm du gène TA26 à partir duquel on peut préparer un ADNc

comprenant la séquence de la figure 3C, ou contre l'ARNm à partir duquel on peut préparer un ADNc comprenant la séquence de la figure 3B; ou est un ADN antisens codant pour un ARN qui est complémentaire de l'ARNm dudit gène TA29, dudit gène TA26 ou dudit gène TA13.

10. Plante selon l'une quelconque des revendications 1 à 6, dans laquelle ledit ADN de stérilité mâle code pour une enzyme qui catalyse la synthèse d'une phytohormone, en particulier l'enzyme codée par le gène 4 de l'ADN-T de Agrobacterium, ou une enzyme codée par le gène 1 et/ou le gène 2 de l'ADN-T de Agrobacterium.

11. Plante selon l'une quelconque des revendications 1 à 10, dans laquelle ledit ADN étranger comprend, en outre:

(e) un premier ADN codant pour un peptide de transit capable de transporter ladite première protéine ou ledit premier polypeptide dans un chloroplaste ou une mitochondrie desdites cellules d'étamines; ledit premier ADN étant dans la même unité de transcription que ledit ADN de stérilité mâle et ledit premier promoteur et entre ledit ADN de stérilité mâle et ledit premier promoteur.

12. Plante selon l'une quelconque des revendications 1 à 11, qui est choisie dans le groupe constitué par le maïs, la pomme de terre, la tomate, le colza, la luzerne, le tournesol, le coton, le céleri, l'oignon, le clou de girofle, le soja, le tabac, les légumes de la famille des crucifères ou la betterave à sucre.

13. Plante selon l'une quelconque des revendications 1 à 12, dans laquelle ledit ADN étranger comprend aussi:

(c) un ADN marqueur codant pour un second ARN, protéine ou polypeptide qui, lorsqu'il est présent au moins dans un tissu spécifique ou dans au moins des cellules spécifiques de ladite plante, rend ladite plante facilement séparable des autres plantes qui ne contiennent pas ledit second ARN, protéine ou polypeptide dans ledit tissu spécifique ou lesdites cellules spécifiques; et

(d) un second promoteur capable de diriger l'expression dudit ADN marqueur au moins dans ledit tissu spécifique ou lesdites cellules spécifiques; ledit ADN marqueur étant dans la même unité de transcription que ledit second promoteur, et étant sous son contrôle.

14. Plante selon la revendication 13, dans laquelle ledit ADN marqueur code pour une protéine inhibant ou neutralisant l'action d'un herbicide.

15. Plante selon la revendication 14, dans laquelle ledit ADN marqueur est un gène de résistance à un herbicide, en particulier un gène conférant une résistance à un inhibiteur de glutamine synthétase tel que la phosphinotricine.

16. Plante selon la revendication 15, dans laquelle ledit ADN marqueur est un gène sfr ou sfrv.

17. Plante selon la revendication 14, dans laquelle ledit ADN marqueur code pour une enzyme cible modifiée pour un herbicide ayant une affinité plus faible pour l'herbicide, en particulier une 5-énolpyruvylshikimate-3 phosphate synthétase comme cible pour le glyphosate ou une glutamine synthétase modifiée comme cible pour un inhibiteur de glutaminme synthéthase tel que la phosphinotricine.

18. Plante selon la revendication 13, dans laquelle ledit ADN marqueur est un gène codant pour une protéine ou un polypeptide conférant une couleur au moins audit tissu spécifique ou auxdites cellules spécifiques, en particulier le gène A1 ou le gène GUS;

ou un gène codant pour une protéine ou un polypeptide conférant une résistance aux maladies ou aux organismes nuisibles, en particulier un gène codant pour une endotoxine Bacillus thuringiensis qui confère une résistance aux insectes ou un gène codant pour un peptide bactéricide qui confère une résistance aux bactéries.

19. Plante selon l'une quelconque des revendications 13 à 18, dans laquelle ledit second promoteur est: un promoteur constitutif, en particulier un promoteur PNOS ou un promoteur POCS; un promoteur susceptible d'être induit par les blessures, en particulier un promoteur TR1' ou TR2', un promoteur qui dirige l'expression du gène sélectivement dans le tissu végétal ayant une activité photosynthétique, en particulier un promoteur SSU; ou un promoteur qui dirige l'expression du gène sélectivement dans les cellules des feuilles, les cellules des pétales ou les cellules des graines, en particulier les cellules de l'enveloppe des graines.

20. Plante selon la revendication 19, dans laquelle ledit second promoteur est un promoteur 35S ou un promoteur SSU.

21. Plante selon l'une quelconque des revendications 13 à 20, dans laquelle ledit ADN étranger comprend, en outre:

(f) un second ADN codant pour un peptide de transit capable de transporter ladite seconde protéine ou ledit second polypeptide dans un chloroplaste ou une mitochondrie d'au moins ledit tissu spécifique ou lesdites cellules spécifiques; ledit second ADN étant dans la même unité de transcription que ledit ADN marqueur et ledit second promoteur et entre ledit ADN marqueur et ledit second promoteur.

22. Plante, qui est une plante susceptible d'être infectée par Agrobacterium et qui contient, incorporé dans le génome nucléaire de la totalité de ses cellules, l'ADN-T de pTTM4 de la figure 6, pTTM6 de la figure 7A, pTTM6A de la figure 7B, pTTM8 de la figure 8, pTVEP1 de la figure 9A, pTVEP2 de la figure 9B, pTVE62 de la figure 10B ou pTVE63 de la figure 10A.

23. Culture des cellules de la plante selon l'une quelconque des revendications 1 à 22.

24. Promoteur du gène TA29 de la figure 3A.

25. ADN qui contient un premier ADN chimérique qui comprend:

(a) un ADN de stérilité mâle codant pour un ARN, protéine ou polypeptide capable, lorsqu'il est produit dans les cellules des étamines de ladite plante, de les tuer ou de les rendre incompétentes pour empêcher la production de gamètes mâles fertiles; et
(b) un premier promoteur qui dirige l'expression génique sélectivement dans les cellules des étamines d'une plante, ladite séquence d'ADN étant dans la même unité de transcription que ledit premier promoteur, et étant sous son contrôle,

à la condition que ledit premier promoteur ne soit pas un promoteur spécifique des microspores et/ou du pollen.

26. ADN selon la revendication 25, dans lequel ledit premier promoteur dirige l'expression dudit ADN de stérilité mâle dans les cellules d'anthère.

27. ADN selon la revendication 26, dans lequel ledit premier promoteur dirige l'expression dudit ADN de stérilité mâle dans les cellules du tapétum ou les cellules épidermiques de l'anthère de ladite plante.

28. ADN selon la revendication 27, dans lequel ledit premier promoteur est le promoteur du gène TA29 de la figure 3A.

29. ADN selon la revendication 27, dans lequel ledit premier promoteur est le promoteur du gène endogène du tabac TA26 codant pour un ARNm à partir duquel on peut préparer un ADNc comprenant la séquence nucléotidique de la figure 3C, le promoteur du gène endogène du tabac TA13 codant pour un ARNm à partir duquel on peut préparer un ADNc comprenant la séquence nucléotidique de la figure 3B, ou un promoteur d'un gène endogène d'une plante codant pour un ARNm spécifique du tapétum susceptible d'être hybridé audit gène TA29 de la figure 3A, audit gène TA26 ou audit gène TA13.

30. ADN selon l'une quelconque des revendications 25 à 29, dans lequel ledit ADN de stérilité mâle code pour une ribonucléase, telle que l'ARNase T1.

31. ADN selon l'une quelconque des revendications 25 à 29, dans lequel ledit ADN de stérilité mâle code pour le barnase.

32. ADN selon l'une quelconque des revendications 25 à 29, dans lequel ledit ADN de stérilité mâle code pour: une ADNase, en particulier une endonucléase, tout spécialement EcoRI; une protéase, en particulier une papaïne, tout spécialement le zymogène de papaïne ou une protéine à activité de papaïne; une glucanase, une lipase, en particulier la phospholipase A2; une peroxydase lipidique; un inhibiteur de paroi cellulaire; une toxine bactérienne; un ribozyme, en particulier le ribozyme contre l'ARNm codé par le gène TA29 de la figure 3A, contre l'ARNm du gène TA26 à partir duquel on peut préparer un ADNc comprenant la séquence de la figure 3C, ou contre l'ARNm à partir duquel on peut préparer un ADNc comprenant la séquence de la figure 3B; ou est un ADN antisens codant pour un ARN qui est complémentaire de l'ARNm dudit gène TA29, dudit gène TA26 ou dudit gène TA13.

33. ADN selon l'une quelconque des revendications 25 à 29, dans lequel ledit ADN de stérilité mâle code pour une enzyme qui catalyse la synthèse d'une phytohormone, en particulier l'enzyme codée par le gène 4 de l'ADN-T de Agrobacterium, ou une enzyme codée par le gène 1 et/ou le gène 2 de l'ADN-T de Agrobacterium.

34. ADN selon l'une quelconque des revendications 25 à 33, qui comprend, en outre:
(e) un premier ADN codant pour un peptide de transit capable de transporter ladite première protéine ou ledit premier polypeptide dans un chloroplaste ou une mitochondrie desdites cellules d'étamines; ledit premier ADN étant dans la même unité de transcription que ledit ADN de stérilité mâle et ledit premier promoteur et entre ledit ADN de stérilité mâle et ledit premier promoteur.

35. ADN selon l'une quelconque des revendications 25 à 34, qui comprend aussi un second ADN chimérique comprenant:

(c) un ADN marqueur codant pour un second ARN, protéine ou polypeptide qui, lorsqu'il est présent au moins dans un tissu spécifique ou dans au moins des cellules spécifiques d'une plante, rend ladite plante facilement séparable des autres plantes qui ne contiennent pas ledit second ARN, protéine ou polypeptide dans ledit tissu spécifique ou lesdites cellules spécifiques; et
(d) un second promoteur capable de diriger l'expression dudit ADN marqueur au moins dans ledit tissu spécifique ou lesdites cellules spécifiques; ledit ADN marqueur étant dans la même unité de transcription que ledit second promoteur, et étant sous son contrôle.

36. ADN selon la revendication 35, dans lequel ledit ADN marqueur code pour une protéine inhibant ou neutralisant l'action d'un herbicide.

37. ADN selon la revendication 36, dans lequel ledit ADN marqueur est un gène de résistance à un herbicide, en particulier un gène conférant une résistance à un inhibiteur de glutamine synthétase tel que la phosphinotricine.

38. ADN selon la revendication 37, dans lequel ledit ADN marqueur est le gène sfr ou sfrv.

39. ADN selon la revendication 36, dans lequel ledit ADN marqueur est un gène codant pour une enzyme cible modifiée pour un herbicide ayant une affinité plus faible pour l'herbicide, en particulier une 5-énolpyruvylshikimate-3 phosphate synthétase comme cible pour le glyphosate ou une glutamine synthétase modifiée comme cible pour un inhibiteur de glutaminme synthéthase tel que la phosphinotricine.

40. ADN selon la revendication 35, dans lequel ledit ADN marqueur est un gène codant pour une protéine ou un polypeptide conférant une couleur au moins audit tissu spécifique ou auxdites cellules spécifiques, en particulier le gène AI ou le gène GUS; ou un gène codant pour une protéine ou un polypeptide conférant une résistance aux maladies ou aux organismes nuisibles, en particulier un gène codant pour une endotoxine Bacillus thuringiensis qui confère une résistance aux insectes ou un gène codant pour un peptide bactéricide qui confère une résistance aux bactéries.

41. ADN selon la revendication 35, dans lequel ledit second promoteur est: un promoteur constitutif, en particulier un promoteur PNOS ou un promoteur POCS; un promoteur susceptible d'être induit par les blessures, en particulier un promoteur TR1' ou TR2'; un promoteur qui dirige l'expression du gène sélectivement dans le tissu végétal ayant une activité photosynthétique; ou un promoteur qui dirige l'expression du gène sélectivement dans les cellules des feuilles, les cellules des pétales ou les cellules des graines, en particulier les cellules de l'enveloppe des graines.

42. ADN selon la revendication 41, dans lequel ledit second promoteur est un promoteur 35S ou un promoteur SSU.

43. ADN selon l'une quelconque des revendications 35 à 42, qui comprend, en outre:
(f) un second ADN codant pour un peptide de transit capable de transporter ladite seconde protéine ou ledit second polypeptide dans un chloroplaste ou une mitochondrie d'au moins ledit tissu spécifique ou lesdites cellules spécifiques; ledit second ADN étant dans la même unité de transcription que ledit ADN marqueur et ledit second promoteur et entre ledit ADN marqueur et ledit second promoteur.

44. ADN, qui est l'ADN-T de pTTM4 de la figure 6, pTTM6 de la figure 7A, pTTM6A de la figure 7B, pTTM8 de la figure 8, pTVEP1 de la figure 9A, pTVEP2 de la figure 9B, pTVE62 de la figure 10B ou pTVE63 de la figure 10A.

45. ADN selon l'une quelconque des revendications 25 à 44, qui est un ADN nucléaire d'une cellule d'une plante ou d'une graine.

46. Vecteur, qui comprend l'ADN selon l'une quelconque des revendications 25 à 44.

47. Cellule d'une plante qui contient l'ADN selon l'une quelconque des revendications 25 à 45.

48. Cellule d'une plante qui contient, intégré de façon stable dans son ADN nucléaire, l'ADN selon l'une quelconque des revendications 25 à 44.

49. Cellule selon la revendication 47 ou la revendication 48, qui peut être régénérée en une plante qui possède une stérilité mâle.

50. Cellule selon l'une quelconque des revendications 47 à 49, dans laquelle ledit ADN comprend un ADN de stérilité mâle qui code pour une ribonucléase.

51. Cellule selon l'une quelconque des revendications 47 à 49, dans laquelle ledit ADN comprend un ADN de stérilité mâle qui code pour le barnase.

52. Cellule selon l'une quelconque des revendications 47 à 51, dans laquelle ledit ADN comprend un premier promoteur qui dirige l'expression dans les cellules d'anthère.

53. Cellule selon l'une quelconque des revendications 47 à 52, dans laquelle ledit ADN comprend un premier promoteur qui dirige l'expression dans les cellules de tapétum.

54. Cellule selon l'une quelconque des revendications 47 à 53, dans laquelle ledit ADN comprend un premier promoteur qui est le promoteur du gène TA29 de la figure 3A.

55. Cellule selon l'une quelconque des revendications 47 à 54, dans laquelle ledit ADN comprend un gène de résistance à un herbicide sous le contrôle d'un second promoteur qui est un promoteur constitutif ou d'un promoteur qui dirige l'expression du gène sélectivement dans le tissu végétal ayant une activité photosynthétique.

56. Cellule selon la revendication 55, dans laquelle ledit gène de résistance à un herbicide confère une résistance à un inhibiteur de glutamine synthétase tel que la phosphinotricine.

57. Cellule selon la revendication 56, dans laquelle ledit gène de résistance à un herbicide est le gène sfr ou sfrv.

58. Cellule selon l'une quelconque des revendications 55 à 57, dans laquelle ledit promoteur est un promoteur 35S ou un promoteur SSU.

59. Plante qui contient l'ADN selon l'une quelconque des revendications 25 à 45.

60. Plante qui contient l'ADN selon l'une quelconque des revendications 25 à 45 dans la totalité de ses cellules.

61. Plante selon la revendication 59 ou 60, qui présente une stérilité mâle.

62. Plante selon l'une quelconque des revendications 59 à 61, qui est une plante hybride.

63. Graine de plante qui contient l'ADN selon l'une quelconque des revendications 25 à 45.

64. Procédé de production d'une plante à stérilité mâle et d'un matériel reproducteur, par exemple de graines de ladite plante à stérilité mâle, qui comprend l'introduction de l'ADN selon l'une quelconque des revendications 25 à 44 dans le génome nucléaire d'une cellule végétale afin d'obtenir une cellule végétale transformée, la régénération de ladite plante à stérilité mâle à partir de ladite cellule végétale transformée et, le cas échéant, l'obtention, à partir de ladite plante à stérilité mâle, dudit matériel de reproduction ou de ladite descendance qui contient ledit ADN étranger.

65. Procédé de production d'une graine d'une plante qui est une plante à stérilité mâle formant des graines, qui comprend:

- la pollinisation croisée i) de plantes selon l'une quelconque des revendications 14 à 22, ou de plantes conte-

nant, intégré de façon stable dans l'ADN nucléaire de la totalité de leurs cellules, l'ADN selon l'une quelconque des revendications 36 à 44, qui sont des plantes formant des graines et présentant une stérilité mâle, dans lesquelles ledit ADN marqueur est un gène conférant une résistance à un herbicide ou un gène codant pour une enzyme cible modifiée pour ledit herbicide, et ii) de plantes à fertilité mâle sans ledit ADN marqueur et ledit second promoteur,

- l'application dudit herbicide aux plantes pour éliminer les plantes à fertilité mâle,
- l'obtention de graines desdites plantes à stérilité mâle pollinisées.

66. Procédé selon la revendication 65, qui comprend l'application dudit herbicide avant ladite pollinisation croisée.

67. Procédé selon la revendication 65, qui comprend l'application dudit herbicide après ladite pollinisation croisée.

68. Procédé selon l'une quelconque des revendications 65 à 67, dans lequel ledit ADN marqueur est un gène capable de conférer une résistance à un inhibiteur de glutamine synthétase tel que la phosphinotricine, en particulier le gène sfr ou sfrv, et qui comprend l'application dudit inhibiteur de synthétase aux plantes.

69. Procédé selon l'une quelconque des revendications 65 à 68, dans lequel les plantes à stérilité mâle contiennent, en plus dudit ADN marqueur, un second ADN marqueur, en particulier un autre gène de résistance à un herbicide ou un gène codant pour une enzyme cible modifiée pour un autre herbicide, intégré de façon stable dans le génome nucléaire de leurs cellules dans le même lieu génétique que ledit ADN de stérilité mâle; et dans lequel lesdites plantes à fertilité mâle utilisées pour la pollinisation contiennent seulement le second ADN marqueur intégré de façon stable dans le génome nucléaire de leurs cellules.

70. Procédé selon l'une quelconque des revendications 65 à 69, dans lequel lesdites plantes à stérilité mâle et lesdites plantes à fertilité mâle sont capables de donner naissance à des plantes hybrides, et qui comprend la récupération des graines hybrides à partir desdites plantes à stérilité mâle.

71. Procédé pour conserver une lignée consanguine de plantes contenant, intégré de façon stable dans l'ADN nucléaire de la totalité de leurs cellules, l'ADN selon l'une quelconque des revendications 35 à 44, ces plantes étant des plantes à stérilité mâle formant des graines qui sont hétérozygotes pour ledit ADN, et dans lesquelles ledit ADN marqueur est un gène conférant une résistance à un herbicide ou un gène codant pour une enzyme cible modifiée pour ledit herbicide, ledit procédé comprenant:

- la pollinisation croisée i) de plantes à stérilité mâle de ladite lignée consanguine, et ii) de plantes à fertilité mâle de ladite lignée consanguine sans ledit ADN marqueur et ledit second promoteur, et, après ladite pollinisation croisée,
- l'obtention de graines à partir desdites plantes à stérilité mâle,
- la culture desdites graines en plantes, et
- l'application dudit herbicide aux plantes pour éliminer les plantes à fertilité mâle.

72. Procédé selon la revendication 71, dans lequel ledit ADN marqueur est un gène capable de conférer une résistance à un inhibiteur de glutamine synthétase, tel que la phosphinotricine, en particulier un gène sfr ou sfrv, et qui comprend l'application dudit inhibiteur de glutamine synthétase aux plantes.

73. Paire de plantes mères pour produire des graines comprenant: a) une plante mère à stérilité mâle contenant l'ADN selon l'une quelconque des revendications 25 à 45, et b) une plante mère à fertilité mâle.

74. Paire de plantes mères selon la revendication 73, dans laquelle ladite plante mère à stérilité mâle et ladite plante mère à fertilité mâle appartiennent à des lignées différentes.

75. Paire de plantes mères selon la revendication 73, dans laquelle ladite plante mère à stérilité mâle et ladite plante mère à fertilité mâle sont dérivées de la même lignée consanguine.

76. Paire de plantes mères selon l'une quelconque des revendications 73 à 75, dans laquelle ledit ADN comprend un ADN de stérilité mâle qui code pour une ribonucléase.

77. Paire de plantes mères selon l'une quelconque des revendications 73 à 75, dans laquelle ledit ADN comprend un ADN de stérilité mâle qui code pour le barnase.

78. Paire de plantes mères selon l'une quelconque des revendications 73 à 77, dans laquelle ledit ADN comprend un premier promoteur qui dirige l'expression dans les cellules d'anthère.

79. Paire de plantes mères selon l'une quelconque des revendications 73 à 78, dans laquelle ledit ADN comprend un premier promoteur qui dirige l'expression dans les cellules de tapétum.

80. Paire de plantes mères selon l'une quelconque des revendications 73 à 78, dans laquelle ledit ADN comprend un premier promoteur qui est le promoteur du gène TA29 de la figure 3A.

81. Paire de plantes mères selon l'une quelconque des revendications 73 à 80, dans laquelle ledit ADN comprend un gène de résistance à un herbicide sous le contrôle d'un second promoteur qui est un promoteur constitutif ou d'un promoteur qui dirige l'expression du gène sélectivement dans le tissu végétal ayant une activité photosynthétique.

82. Paire de plantes mères selon la revendication 81, dans laquelle ledit gène de résistance à un herbicide confère une résistance à un inhibiteur de glutamine synthétase tel que la phosphinotricine.

83. Paire de plantes mères selon la revendication 82, dans laquelle ledit gène de résistance à un herbicide est le gène sfr ou sfrv.

84. Paire de plantes mères selon l'une quelconque des revendications 81 à 83, dans laquelle ledit promoteur est un promoteur 35S ou un promoteur SSU.

FIG.1

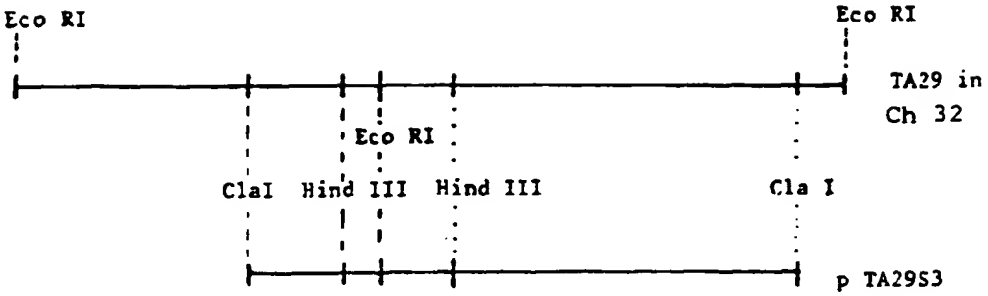
Eco RI
Eco RI
TA29 in
Ch 32
Eco RI
ClaI
Hind III
Hind III
Cla I
p TA29S3

Fig. 2

```
                                    27                                  54
CAA TCC GCT AGA CTA TAC CGT TGC AAG CCA GGG CCA AAT ATG TGT GAC AGT AAA
Q   S   A   R   L   Y   R   C   K   P   G   P   N   M   C   D   S   K

                                    81                                  108
GAC TGT AAT GAG CTT CTC CTA CAC TTT GTT TTC CCA ATG CAA GAC AAA CAT GAC
D   C   N   E   L   L   L   H   F   V   F   P   M   Q   D   K   H   D

                                    135                                 162
AAT AAA CAA GAA CAT CTA AGA TAT GGA GGA CGC CGA GGT ATA GGT CTC ACT GTG
N   K   Q   E   H   L   R   Y   G   G   R   R   G   I   G   L   T   V

                                    189                                 216
GGA GGA GTT GGC GGT TTT GGA ATT GGT TTT GGT GCT TGG GGT GGT GGT GGT GGC
G   G   V   G   G   F   G   I   G   F   G   A   W   G   G   G   G   G

                                    243                                 270
GGA GGA GGT GGT GGT TCT GAT GCC CCT GGT TGT AGT AAC GAT GGC TGT GAC CCT
G   G   G   G   G   S   D   A   P   G   C   S   N   D   G   C   D   P

                                    297                                 324
GGT TTT GGC TGT CCC CCG GGC TGT GGT TAT GCA TGT CCT GCC AAC AAT CCT AGT
G   F   G   C   P   P   G   C   G   Y   A   C   P   A   N   N   P   S

                                    351
GGA GGA ATA ACT GAA TTC CAT ATC TCA GGA TTG TTG GCA
G   G   I   T   E   F   H   I   S   G   L   L   A
```

Figure 3 A (continued)

```
                                                    cDNA clone TA29  -->   ACAATCC
CCATGTGTGTCCGGCCGATATTCCTACTGGAGGAATGACTGAATCCAAAATCACAGGAATATCACAATCC 1946
 Y  V  C  P  A  D  I  P  T  G  G  M  T  E  S  K  I  T  G  I  S  Q  S

GCTAGACTATACCGTTGCAAGCCAGGGCCAAATATGTGTGACAGTAAAGACTGTAATGAGCTTCTCCTAC
GCTAGACTATACCGTTGCAAGCCAGGGCCAAATATGTGTGACAGTAAAGACTGTAATGAGCTTCTCCTAC 2016
A  R  L  Y  R  C  K  P  G  P  N  M  C  D  S  K  D  C  N  E  L  L  L  H

ACTTTGTTTTCCCAATGCAAGACAAACATGACAATAAACAAGAACATCTAAGATATGGAGGACGCCGAGG
ACTTTGTTTTCCCAATGCAAGACAAACATGACAATAAACAAGAACATCTAAGATATGGAGGACGCCGAGG 2086
  F  V  F  P  M  Q  D  K  H  D  N  K  Q  E  H  L  R  Y  G  G  R  R  G

TATAGGTCTCACTGTGGGAGGAGTTGGCGGTTTTGGAATTGGTTTTGGTGCTTGGGGTGGTGGTGGTGGC
TATAGGTCTCACTGTGGGAGGAGTTGGCGGTTTTGGAATTGGTTTTGGTGCTTGGGGTGGTGGTGGTGGC 2156
 I  G  L  T  V  G  G  V  G  G  F  G  I  G  F  G  A  W  G  G  G  G  G

GGAGGAGGTGGTGGTTCTGATGCCCCTGGTTGTAGTAACGATGGCTGTGACCCTGGTTTTGGCTGTCCCC
GGAGGAGGTGGTGGTTCTGATGCCCCTGGTTGTAGTAACGATGGCTGTGACCCTGGTTTTGGCTGTCCCC 2226
G  G  G  G  G  S  D  A  P  G  C  S  N  D  G  C  D  P  G  F  G  C  P  P
                                                    EcoRI
CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATT
CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATT 2296
  G  C  G  Y  A  C  P  A  N  N  P  S  G  G  I  T  E  F  H  I  S  G  L

 <--  end cDNA clone TA29
ATCACGATTCGATGGACCTTACAGATGTAGGCCAGATATGTGTGAAAGTGAAGATTGTAATGAACTTCTT 2366
 S  R  F  D  G  P  Y  R  C  R  P  D  M  C  E  S  E  D  C  N  E  L  L

CTACACTTTGTTTCTCCAATGCAACACAAACATGAGAACCGACATGATCATATAGTAGAAAGGAGTGATG 2436
L  H  F  V  S  P  M  Q  H  K  H  E  N  R  H  D  H  I  V  E  R  S  D  E

AGGAGGAAGCGCATCATCAGTCAAAGCAGCATAAAGACGAAGACATCATAAACTAGGCTCTCCCACAAAC 2506
  E  E  A  H  H  Q  S  K  Q  H  K  D  E  D  I  I  N  *

CAAAAAAAAAAGGAACTATATATGTAGCTTCAGCCAAAAAACTGTATACACTGTCTAAGAATACTCACTTC 2576

CAACGAACTTAAATAAAACTAGTTTACAGTGGATTGGGATATAATCAGTTGGACAATTTGCTAAACCTCC 2646

TCATGCACTGTAAAAATAGACTTGCTACTAGTATTTGGAATATAATGCTGAATATATTTGTTGTTACTTT 2716

GCCTAATGTCAATCAGCATTCAGCAATTTCTCTGTAGTTAGAAAATGAAAGGAAGAATCAGGAAACTCAT 2786

ATTTAAAGGATGAAATAATTTAAAGATCGCGAAGCAGTCACAATTTAATAGTACCAGGAAAATAATCTAT 2856

AGGAATCACAGAACTTTTTGATTTATCAAATTAAGGAAGCAAACTGGGAAAATGTGAAATGAATGAACAT 2926

AATGCTGAAAGCTATTGATCAGATGATTGGATTGATTTCGTAGGAGCAACATATGATTTAAGATTATTTC 2996

AACAAGATGGCCATAAAGTAGCATATCATTTGTAATTTAACATTATTACACTCAAACTCAGGAAGATTGT 3066

CAATTTACCCTCAAAACAAAGTTTTAAGCCTTCAGTCTCCTTCAACCACAGTGGCACCTGCCCAATTGGC 3136

AGCACTTCCCCCGGCGTGGAATGCTGTGGAGTTTGGTGTACAAATCCACCTGGAAAATCACAGCATTGATG 3206
                                                          HindIII
TTTCCTTCATCATCTTCGCGTGCAATTGCTTTTACTTTGTGCAGTGGATGATCAAAGCTT 3266
```

Figure 3 B

```
  1 aaagtctttacttaaagaaattagctaaaatggtagctccaaaatggtctttcatttcttttatgattttgc

 73 taagcttagcaatatgctctggccagcctgttacctttgatgcaattaaggctaaggaagctgatcatgaca

145 acctcaaagctcacactctaagtaatatcgacaccaaaggctttggaggaggcggtggatttggcattggtg

217 gtgtttgggccggaggtggtggtggtggttctgacgccctaactacggttataaccctggctgcagta

289 tccgtggttgcactgtccctggctttggtttcctacctaatcctggttttggtgttccagtctattcccctg

361 gttgtggctatgtgtgtccagccgatatttctgctgaaggaatgactgaatccaaaatcacaggaatatcAg
                                                                          |
  1                                                                       Ac

433 AATCgGCTAGACcATAtOGaTGCAgGCCtGGGgCAAATATGTGTGgCAGTAAAGAtTGTAATGAGCTTCTCC
    |||| ||||||| ||| || |||| ||| ||| ||||||||||||| ||||||||| ||||||||||||||||
  8 AATCoGCTAGACtATAcOGtTGCAaGCCaGGGcCAAATATGTGTGaCAGTAAAGAcTGTAATGAGCTTCTCC

505 TACACTTTGTTTTCCCcATGCAAGACAAACATGAgAATAAACAAGAAtATCTAAGATATGGAGGACGtCGAG
    |||||||||||||||| |||||||||||||||| |||||||||||| |||||||||||||||||| ||||
 75 TACACTTTGTTTTCCCaATGCAAGACAAACATGAcAATAAACAAGAAcATCTAAGATATGGAGGACGcCGAG

577 GTATAGGTCTCAgTGTGaGtGaAtcTaGtGGTTTTGGAATTGGTTTTGGTGCTcgggGtGGTGGTGGTGGcG
    |||||||||||| |||| | | |  | | ||||||||||||||||||||||||    | |||||||||| |
147 GTATAGGTCTCAcTGTGgGaGgAgtTgGcGGTTTTGGAATTGGTTTTGGTGCT   tGgGGTGGTGGTGGtG

649 GCGGAGGAGGgGGTGaTTCTaATGCCCCTGGcTtTgaTAtCcccGGaTtTaACCCcGGcTTTGGCTGTCCCt
    |||||||||| |||| |||| |||||||||| | |  || |  || | | |||| || ||||||||||||
216 GCGGAGGAGGtGGTGgTTCTgATGCCCCTGGtTgTagTAaCgatGGcTgTgACCCtGGtTTTGGCTGTCCCc

721 CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATTaT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
238 CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATTgT

793 cacgAaacaatggaccttacagatgtaggccagatatgtgtgagagtgaagattgtaatgaacttctactac
      |
360 tggcAg

865 actttgtttctccaaagcaacacaaacacgagaacgacatgatcatacagtagaaagaaatgaagaggagg

937 aagctcatcatcagtcaaagcatcataaagacgaagacatcataaactag
```

SEQUENCE OF cDNA TA26

```
        10         20         30         40         50         60         70
CGCAGGGGGG GGGGGGGGGG GGGGGGCAAA GATGTCAATA ACTTCCAAGT TCTTGCTAGT TATGTCCCTA

        80         90        100        110        120        130        140
GGACTAATAG TTTTTACCAC ATTTTCACTT GCTGATCAAC ACTACCAATC TACCAAACAT GAGCTTGGAC

       150        160        170        180        190        200        210
GTTCTGATAC TAATCAGCTA AACATGAATG GTTACTTAGC CATGGAACCA GCACCACCAG ACCTTGAGCA

       220        230        240        250        260        270        280
AGAAGGGCAT ATGTGGCGCT TGAACGACGA CTCGATCGCC ATGGAACCAG CACCACCAGA CCTTGAGCAA

       290        300        310        320        330        340        350
GAAGTGCATA TGTGGCGCTT GAACGACGAC TCGATCGCCA TGGAACCAGC ACCAAGGTTT GAGCTAGAAG

       360        370        380        390        400        410        420
GGCAGAAGCA ACATGAGCAT GAGTCACACT TGAGGCTAGT AACTTAGAAA ACATGATTAG CACCAGAATA

       430        440        450        460        470        480        490
GAATTAACTT GGAAGATGGT GGATTATTGT ACTATAGTCC CTTATTCTAA GTTGTGGATC AATAATAAAG

       500        510        520        530        540        550        560
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C
```
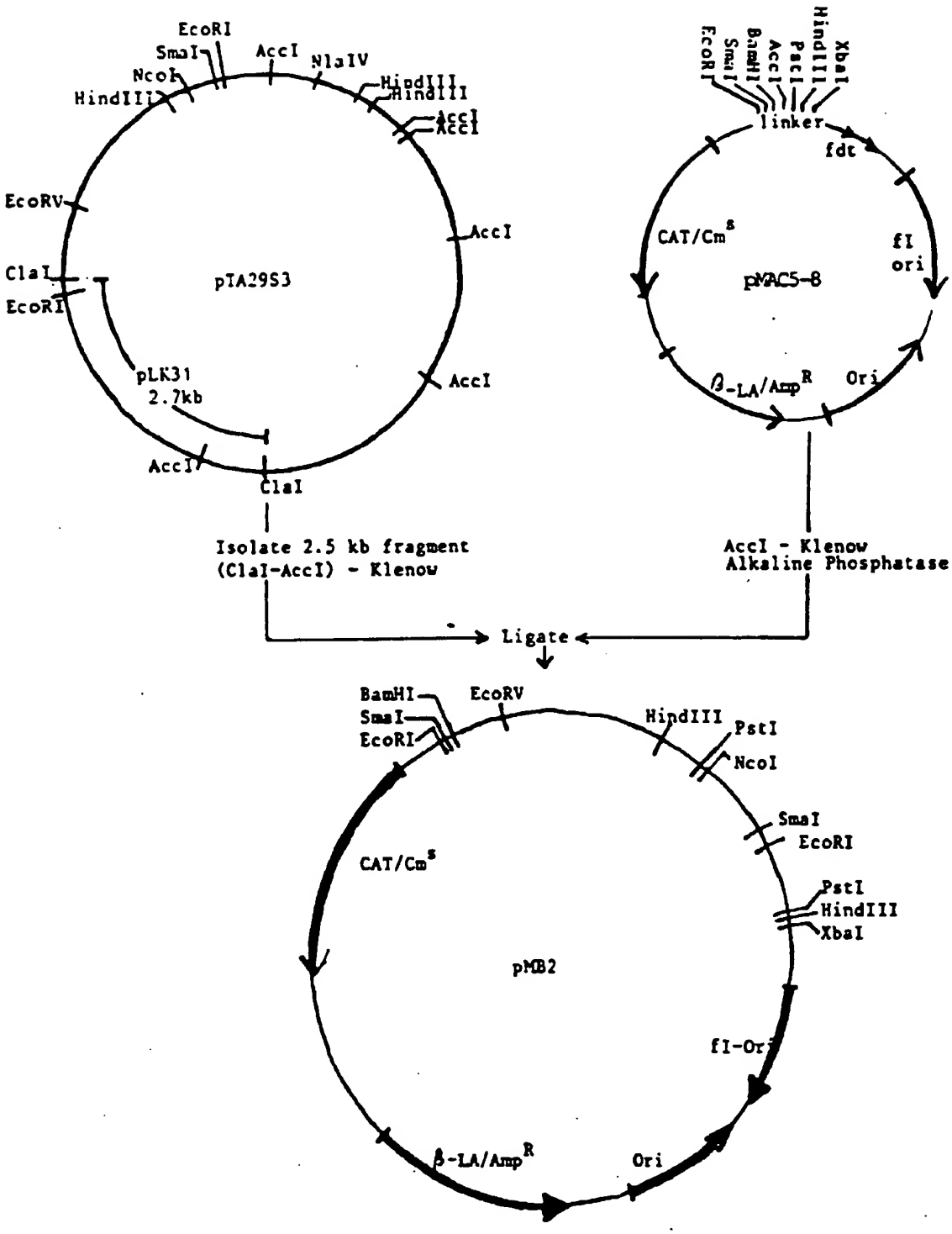

FIGURE 3C

Fig. 4A


EcoRI
SmaI
AccI
NlaIV
NcoI
HindIII
HindIII
HindIII
AccI
AccI
EcoRV
AccI
ClaI
EcoRI
pTA2953
pLK31
2.7kb
AccI
AccI
ClaI
EcoRI
SmaI
BamHI
AccI
PstI
HindIII
XbaI
linker
fdc
CAT/Cm^R
pMAC5-8
fI
ori
β-LA/Amp^R
Ori
Isolate 2.5 kb fragment
(ClaI-AccI) - Klenow
AccI - Klenow
Alkaline Phosphatase
Ligate
BamHI
SmaI
EcoRI
EcoRV
HindIII
PstI
NcoI
SmaI
EcoRI
CAT/Cm^R
PstI
HindIII
XbaI
pMB2
fI-Ori
β-LA/Amp^R
Ori

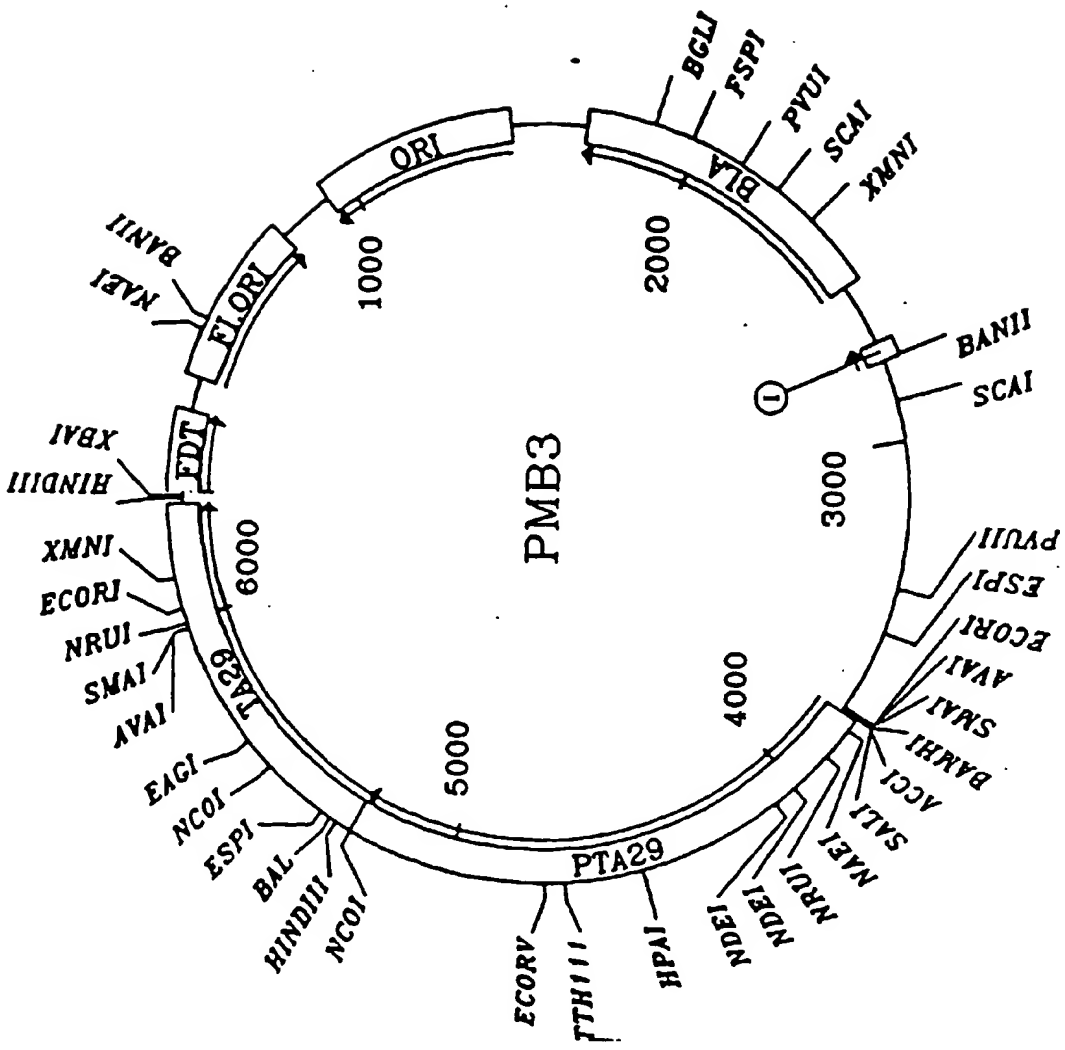


FIG.4 B.

LEGEND

① π

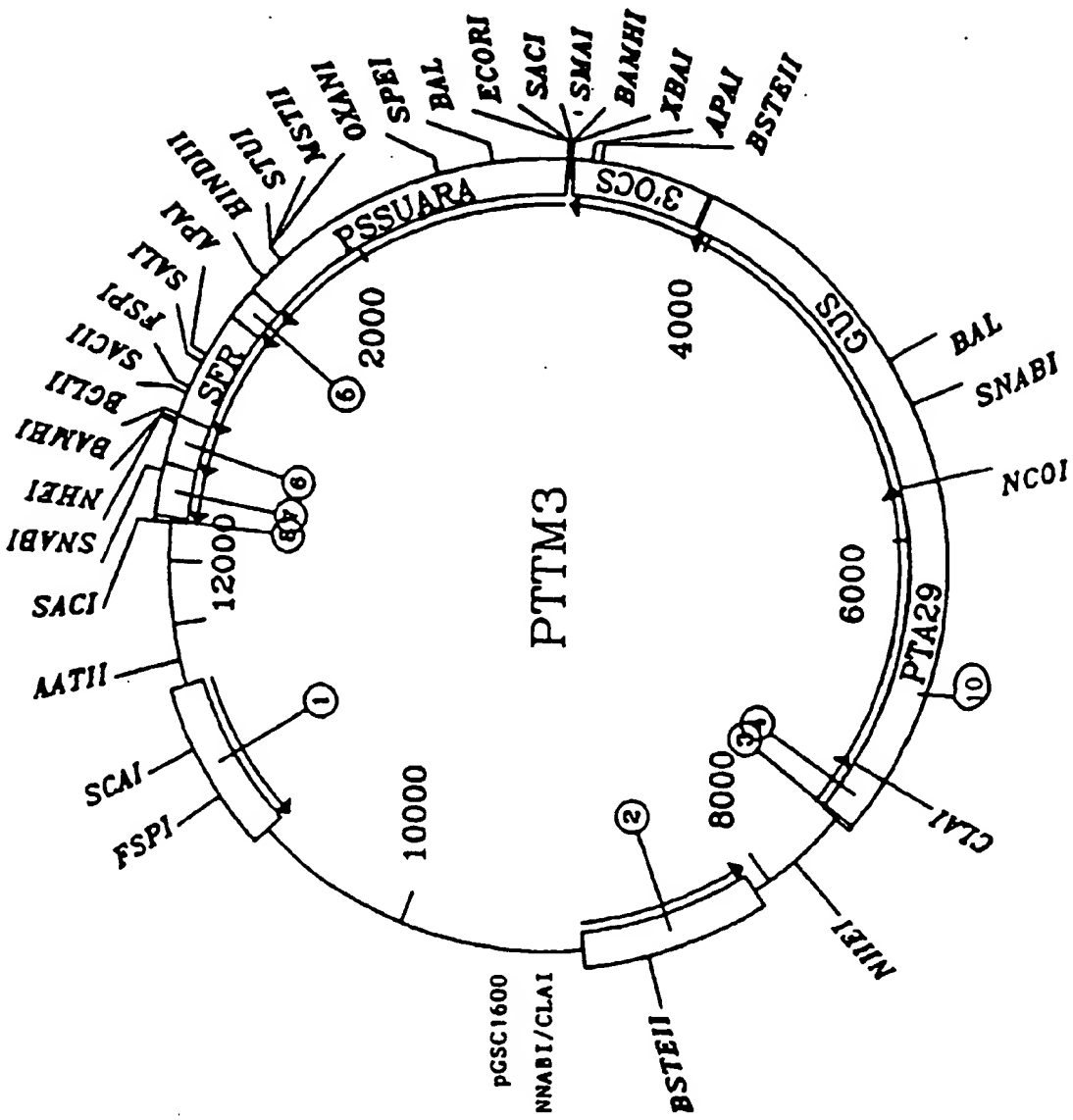


FIG. 5

LEGEND

① BETA-LACTAMASE
② SM-SP-AD-TRANSF.
③ LB
④ T-DNA
⑤ TP
⑥ 3'END T7
⑦ T-DNA
⑧ RB
⑩ PTA29S3/pMB3

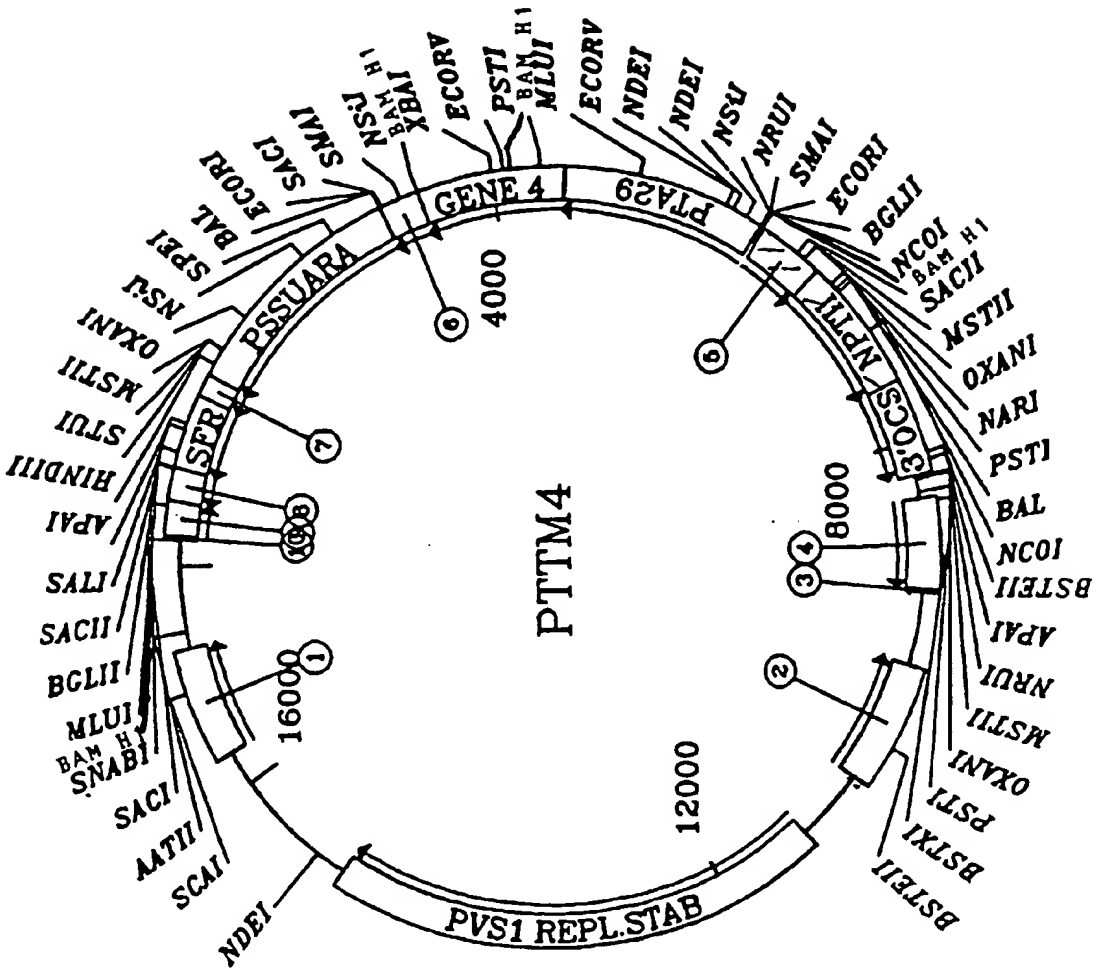


FIG. 6

LEGEND

① BETA-LACTAMASE
② SM-SP-AD TRANSF.
③ LB
④ T-DNA
⑤ PNOS
⑥ 3'NOS
⑦ TP
⑧ 5'END T7
⑨ T-DNA
⑩ RB

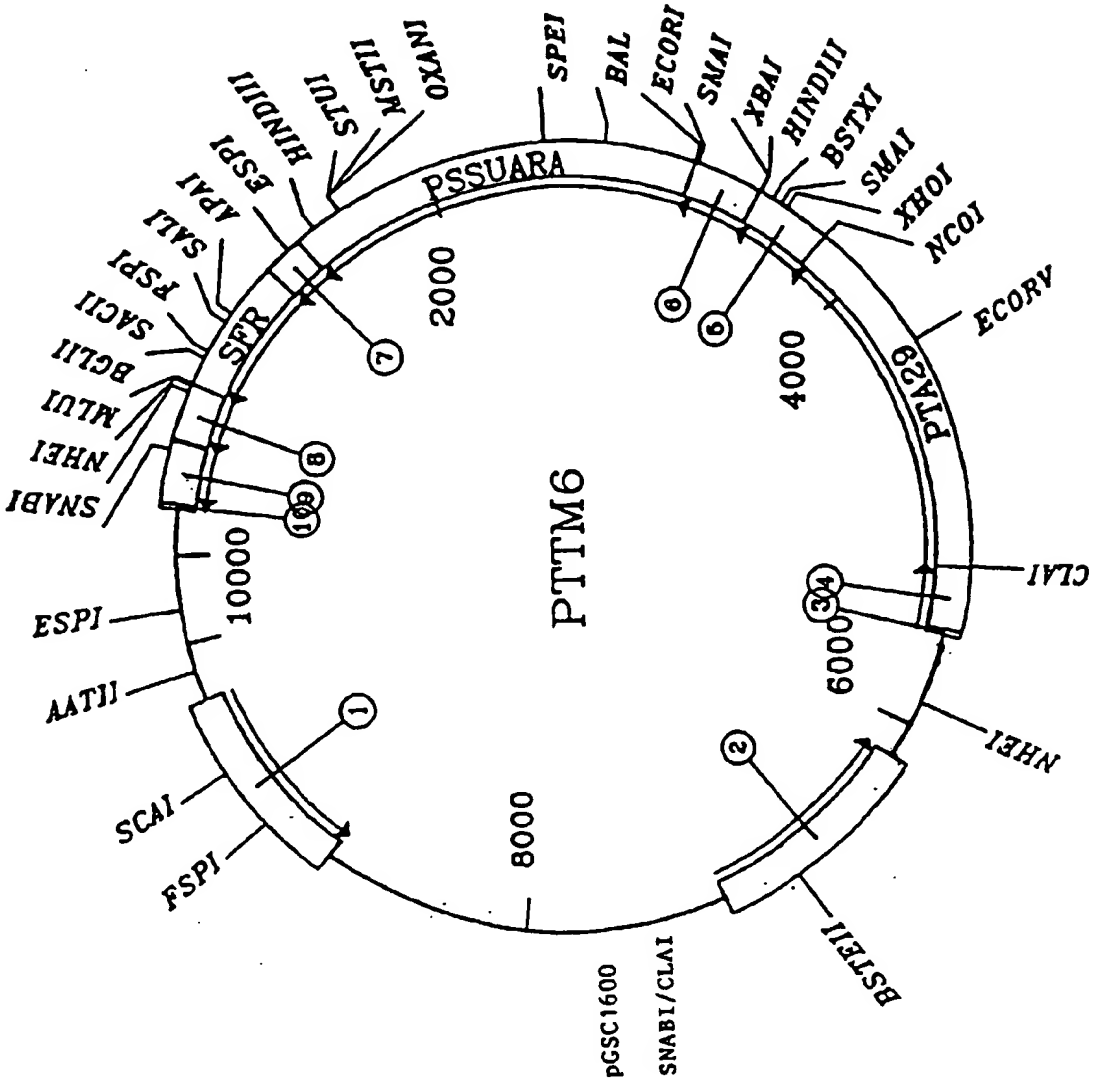


FIG. 7 A

LEGEND

① BETA-LACTAMASE
② SU-SP-AD.TRANSF.
③ LB
④ T-DNA
⑤ RNASE T1
⑥ 3'END NOS
⑦ TP
⑧ 3'END T7
⑨ T-DNA
⑩ RB

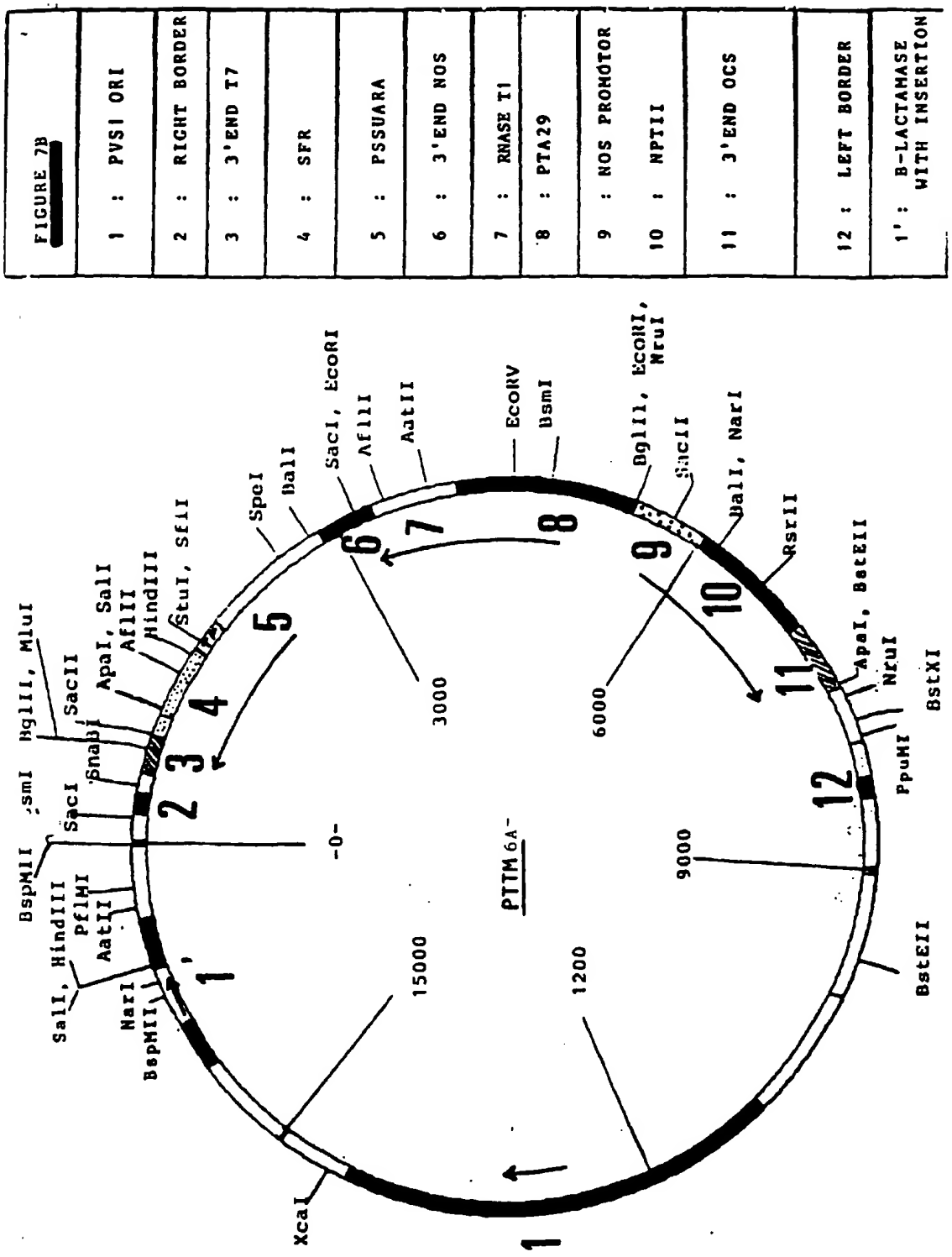


FIGURE 7B
1 : PVS1 ORI
2 : RIGHT BORDER
3 : 3'END T7
4 : SFR
5 : PSSUARA
6 : 3'END NOS
7 : RNASE T1
8 : PTA29
9 : NOS PROMOTOR
10 : NPTII
11 : 3'END OCS
12 : LEFT BORDER
1' : B-LACTAMASE WITH INSERTION
PTTM6A-
-0-
3000
6000
9000
12000
15000
BglII, MluI
SacII
ApaI, SalI
AflII
HindIII
StuI, SfiI
SpeI
BalI
SacI, EcoRI
AflII
AatII
EcoRV
BsmI
BglII, EcoRI, MruI
SacII
BalI, NarI
BsrII
ApaI, BstEII
NruI
BstXI
PpuMI
BstEII
XcaI
BspMII
PflMI
AatII
NarI
SalI, HindIII
SacI
BspMII

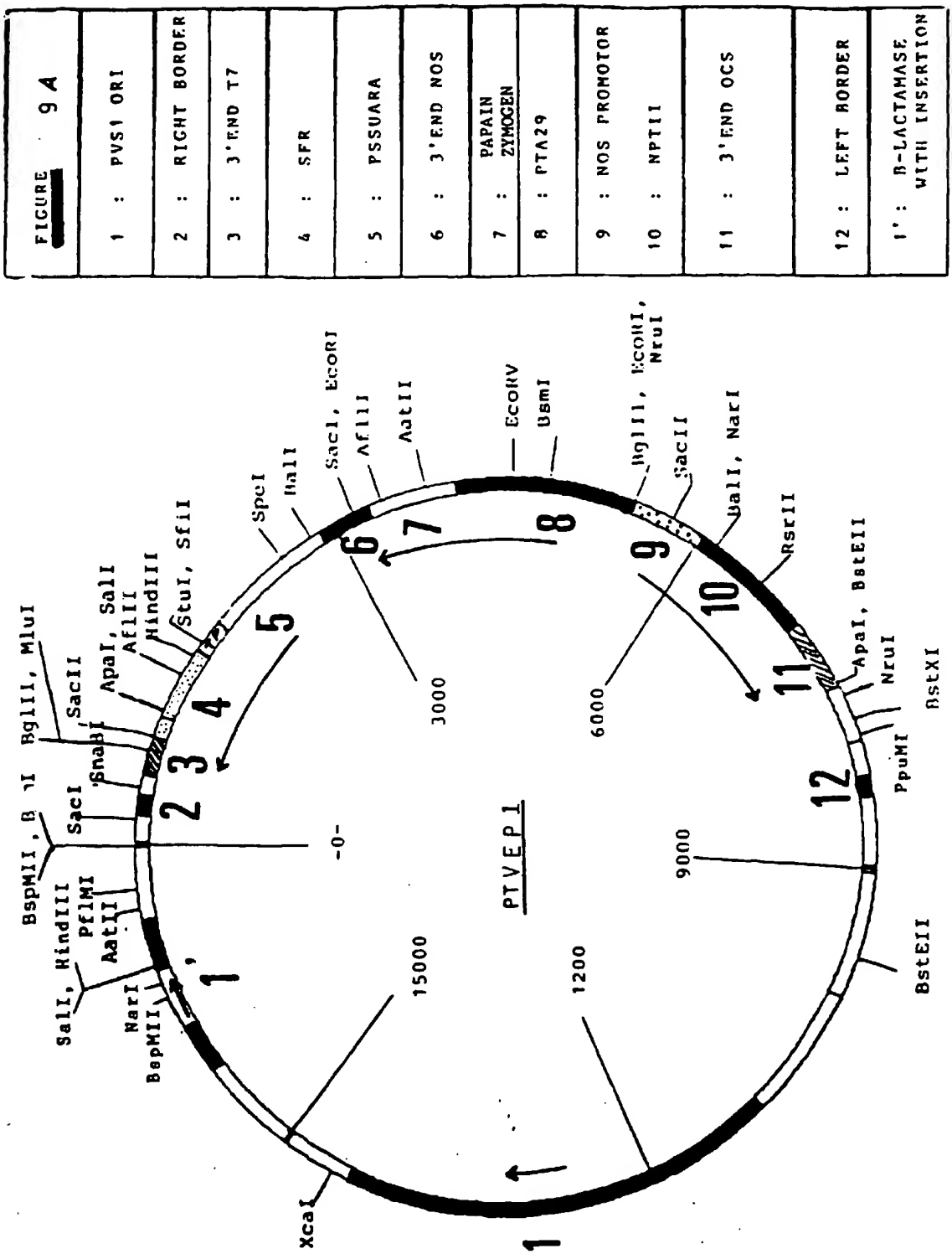


FIGURE 9A	
1 : PVS1 ORI	
2 : RIGHT BORDER	
3 : 3'END T7	
4 : SFR	
5 : PSSUARA	
6 : 3'END NOS	
7 : PAPAIN ZYMOGEN	
8 : PTA29	
9 : NOS PROMOTOR	
10 : NPTII	
11 : 3'END OCS	
12 : LEFT BORDER	
1' : B-LACTAMASE WITH INSERTION	

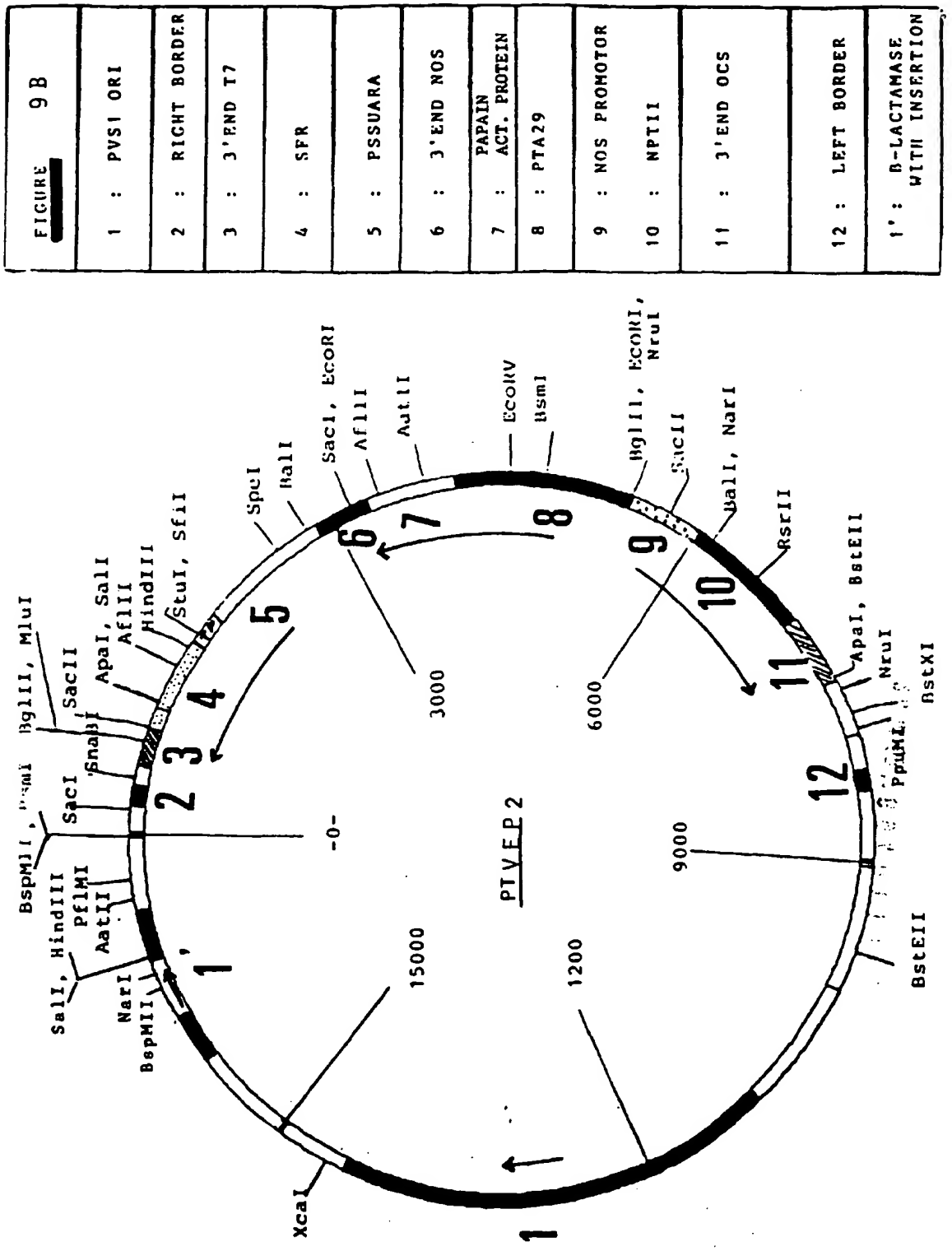


FIGURE 9 B
1 : PVS1 ORI
2 : RIGHT BORDER
3 : 3'END T7
4 : SFR
5 : PSSUARA
6 : 3'END NOS
7 : PAPAIN ACT. PROTEIN
8 : PTA29
9 : NOS PROMOTOR
10 : NPTII
11 : 3'END OCS
12 : LEFT BORDER
1' : B-LACTAMASE WITH INSERTION
PTVEP2
-0-
3000
6000
9000
12000
15000
BglII, MluI
SacII
ApaI, SalI
AflII
HindIII
StuI, SfiI
SpeI
BalI
SacI, EcoRI
AflII
AatII
EcoRV
BsmI
BglII, EcoRI, NruI
SacII
BalI, NarI
RsrII
ApaI, BstEII
NruI
BstXI
BstEII
BspMII
SacI
PflMI
AatII
SalI, HindIII
NarI
BspMII
XcaI

FIGURE 10 A

1 : PVS1 ORI
2 : PBR ORI
3 : RIGHT BORDER
4 : 3' END GENE 7
5 : SFR GENE WITH TRANSIT PEPTIDE
6 : PSSUARA
7 : 3' END NOS
8 : EcoRI
9 : PTA29
10 : NOS PROMOTOR
11 : NPTII
12 : 3' END OCS
13 : LEFT BORDER
14 : METHYLASE RI
15 : SM/SP

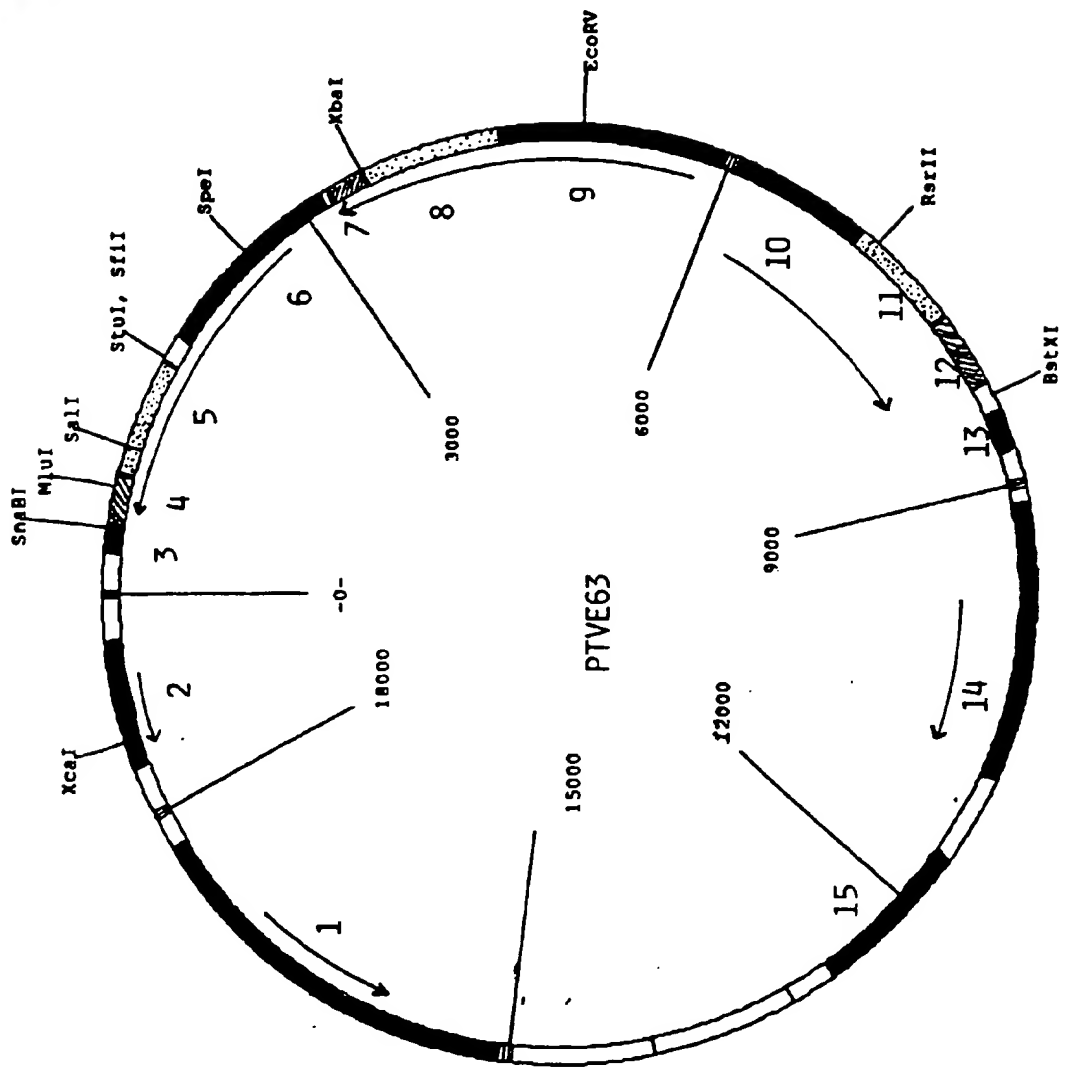
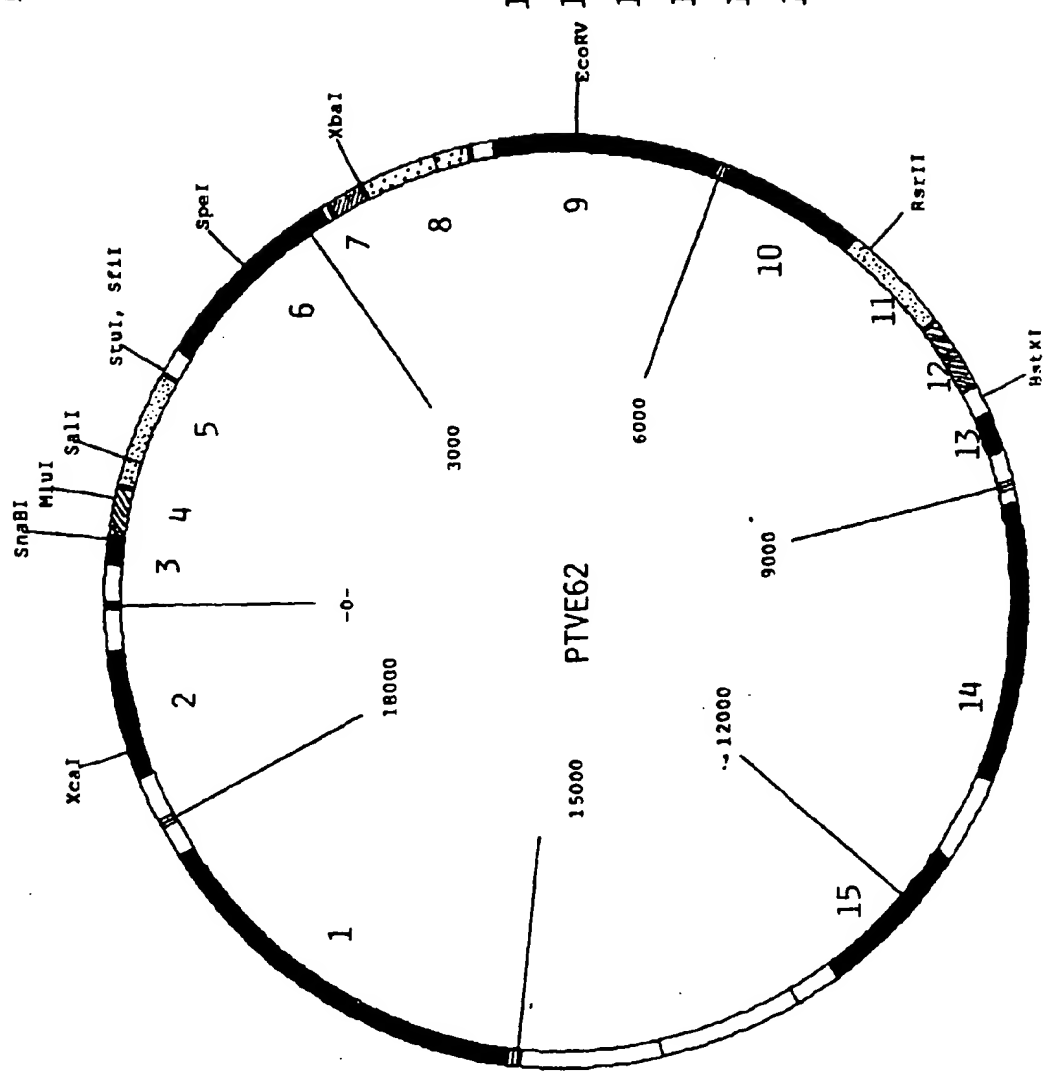
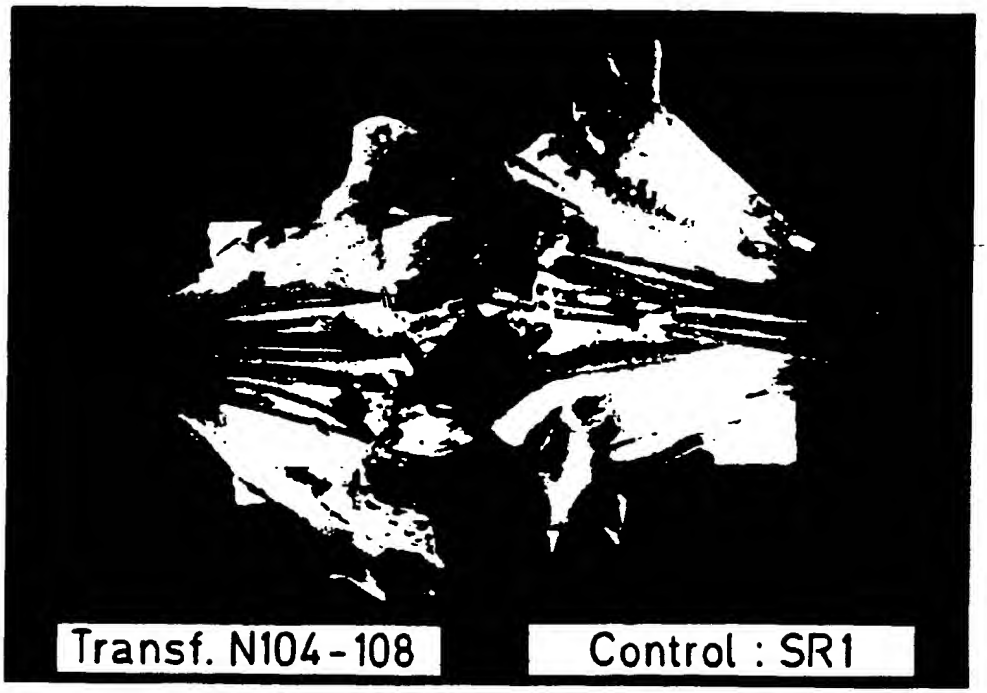


FIGURE 10 B

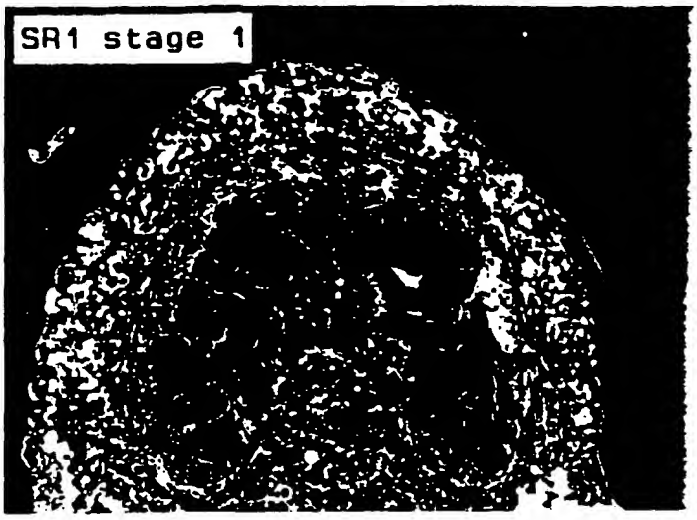
1 : PVS1 ORI
2 : PBR ORI
3 : RIGHT BORDER
4 : 3' END GENE 7
5 : SFR GENE WITH TRANSIT PEPTIDE
6 : PSSUARA
7 : 3' END NOS
8 : ECORI WITH TRANSIT PEPTIDE
9 : PTA29
10 : NOS PROMOTOR
11 : NPTII
12 : 3' END OCS
13 : LEFT BORDER
14 : METHYLASE RI
15 : SM/SP





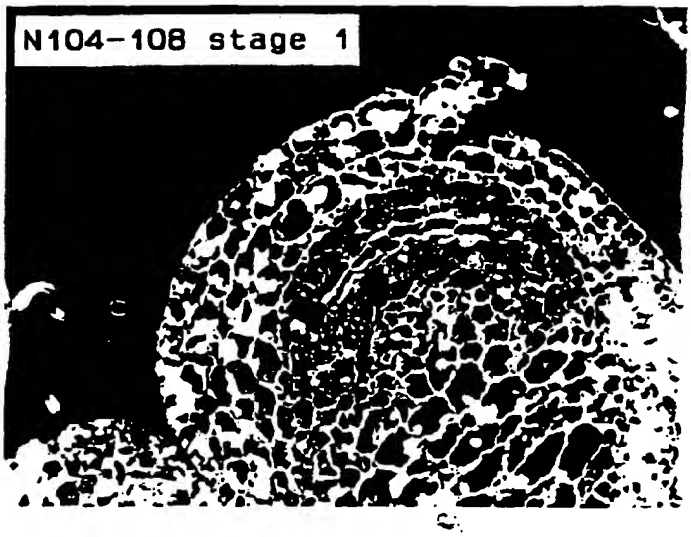
TRANSFORMED SR1 CONTROL SR1

Fig. 11



1 SR1 stage 1
anther cross section
thick tapetum around
pollen sac

pollen stage:tetrad form.
X250 d.f.



2 N104-108 stage 1
anther cross section
thin layer of tapetum
no pollen sac

no pollen visible
X250 d.f.

Fig. 12